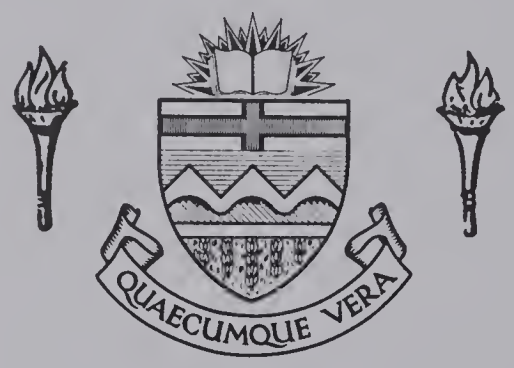


The University of Alberta
Printing Department
Edmonton, Alberta

LIFE IS THE ART OF DRAWING SUFFICIENT

CONCLUSIONS FROM INSUFFICIENT PREMISES

Samuel Butler

THE UNIVERSITY OF ALBERTA

PERFUSION STUDIES IN PRESERVATION OF THE ISOLATED CANINE LUNG

By

© DENNIS WILLIAM JIRSCH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL, 1970

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Perfusion Studies in Preservation of the Isolated Canine Lung" submitted by Dennis William Jirsch, in partial fulfillment of the requirements for the degree of Master of Science (Surgery).

Abstract

Before transplantation can become a widely applicable means of treating pulmonary disease, adequate short term lung preservation methods must be established. This problem has been approached in two ways. The empiric combination of hyperbaric oxygen (15-30 psi) and hypothermia has been shown to permit lung storage for 24 hours with successful reimplantation or allografting. Lungs stored in this fashion have been evaluated weeks or months after surgery and have demonstrated adequate function. Other workers have attempted to preserve lungs with artificial extracorporeal circulation. This has proven difficult and routinely successful perfusions of the isolated lung have not been reported for periods longer than 6 hours.

The problems involved in lung preservation have not been widely investigated. The object of this work was to implement an organ perfusion apparatus to establish the functional adequacy of lung storage with hypothermia and hyperbaria and, in addition, to investigate the conditions necessary for successful lung perfusion preservation.

Left lower canine pulmonary lobes were stored under hypothermic hyperbaric conditions for 24 hours and were then subjected to normothermic blood perfusion. Non-stored control lobes functioned well for at least four hours but preserved lobes deteriorated rapidly with gross pulmonary edema after only 60 to 90 minutes of perfusion. Lobes stored in this manner were unsuitable for transplant purposes since immediate functional adequacy is necessary.

In an attempt to extend the limits of functional lung preservation by perfusion, left lower lobes were placed in a perfusion system which closely simulated the normal intrathoracic hemodynamics and ventilation. The donor animal was incorporated into the perfusion circuit as a convenient deoxygenator and means of maintaining the normal metabolic environment. These conditions permitted routine functional preservation of isolated pulmonary lobes for 12 to 18 hours.

Clinical organ preservation cannot include a donor subject to maintain perfusate composition. For this reason biochemical changes were investigated in lung perfusions excluding a donor animal. Rapid depletion of blood glucose with accumulation of metabolic acids occurred over a 5 hour perfusion interval and, in addition, vasoactive substances were released from the isolated perfused lung in significant quantities.

The hemodynamic and functional effects of positive pressure ventilation of the lung were investigated in a further series since many workers have reccommended positive pressure ventilation in isolated lung perfusion. Pulmonary lobes were evaluated during perfusion with alternate negative and positive pressure ventilation at equal tidal volumes and comparable conditions of flow, functional residual capacity and venous pressure. Positive pressure ventilation was found to be associated with increased pulmonary vascular resistance, diminished compliance and less effective gas exchange. Function and hemodynamics were not similarly compromised with physiologic intermittent negative pressure ventilation.

ACKNOWLEDGEMENTS

The author is deeply indebted for the assistance given him from many sources. Special thanks are due to:

Dr. C. M. Couves, my supervisor, for consistent encouragement and guidance throughout this study.

Dr. R. L. Fisk for advice and helpful criticism

Dr. J. C. Russel for willing assistance regarding statistics and interpretation of biochemical data.

Mr. J. McCallum for excellent technical assistance and unusual enthusiasm.

Miss M. Lear, Mrs. M. McCubbin and Miss A. Hunter for many long hours spent with numerous laboratory procedures.

Mr. R. Orchard and Mr. A. Wells for cheerful surgical assistance.

Mrs. J. Larsen for complete and dedicated secretarial help, far beyond the call of duty.

Mrs. M. Stewart, Mrs. R. Harringa and Miss G. Emery for typing segments of this paper on very short notice.

Medical Research Council of Canada, for important financial aid, both for the Project and the Fellowship.

My wife Jacqueline, for daily optimism and encouragement.

TABLE OF CONTENTS

L I S T O F T A B L E S

LIST OF FIGURES

CHAPTER I

A. WHOLE ORGAN PRESERVATION

Current interest in organ preservation is due to the remarkable advances in organ transplantation during the past decade. The importance of matching transplants by histocompatibility typing is now apparent since it has been shown that the probability of finding suitable organ donors in the cadaver population is minute.[1] Adequate means of organ preservation for even the short periods of 12 to 24 hours would result in improved matching of donor and recipient and would increase the applicability of transplantation in a wide variety of disease states.

Work in organ preservation has proceeded along two main lines. Researchers have either attempted to increase the resistance of the isolated organ to anoxic injury or have attempted to simulate normal physiologic conditions with the provision of abundant essential nutrients.

1. REDUCTION OF METABOLIC NEED

a. Hypothermia

Hypothermia has long been known to confer a measure of protection on anoxic organs and was originally shown to be helpful for anoxic kidneys by Bogardus and Schlosser in 1956.[2] The protection is due to a general depression of metabolism with diminished oxygen requirements and accumulation of toxic products. The depression is exponentially related to temperature[3] with oxygen consumption being reduced to 16% at 20°C and to 5% at 10°C. Though hypothermia has found general acceptance in minimizing organ damage in the interval from donor to transplant and has even protected anoxic organs for periods up to 12 hours, it has proven more useful when combined with other methods.

b. Freezing

Whole organ freezing is an extension of the hypothermia concept and seems the most promising method for long-term organ storage. Problems in the field of cryobiology relate to the fact that ice crystals injure cells either mechanically or osmotically with the hypertonic salts which develop as ice crystals precipitate out. Numerous agents termed cryoprotectants, including glycerol and dimethyl sulfoxide, can minimize damage due to freezing by virtue of their colligative or water binding properties, and have experimentally permitted storage of frozen sperm[4] and red cells[5] for years. The problems of cryoprotective addition, heat transfer gradients during thawing and varying cellular requirements have thus far precluded successful thorough freezing of whole mammalian organs.

c. Hyperbaric Pressure

In 1964 Manax et al.[6] first reported the use of hyperbaric oxygen combined with hypothermia for the preservation of kidneys. This technique allowed canine kidneys stored for 24 hours to maintain life after reimplantation and delayed contralateral nephrectomy. Oxygen at 2 to 3 Atmospheres pressure in conjunction with hypothermia of 4 to 5^{o}C has since been applied to the storage of heart, lung and bowel segments[7-12] for 24 hour intervals and results have indicated that the combination of hyperbaroxia and hypothermia is consistently more effective than either alone. The use of hyperbaric oxygen is empiric since the exact mode of function is unknown. Hangaard[12] and Dickens[13] have demonstrated that one effect of hyperbaric oxygen in vitro is the inhibition of sulfhydryl containing enzymes which could be detrimental to the anoxic cell; others point out that it may be the pressure of the gas itself which is important since even under high pressure oxygen diffusion from the

surface of whole organs could affect only a thin rim of peripheral tissue. Hyperbaric nitrogen and helium have been used in renal preservation experiments with beneficial effect.[14] Recently hyperbaric oxygen has been combined with hypothermic perfusion of livers,[15] kidneys[16] and hearts[17] but successful preservation longer than 24 hours has still only rarely been reported.

d. Pharmacologic Agents

A number of drugs can experimentally slow tissue metabolism but have thus far proven much less effective than hypothermia in protecting anoxic organs. Antithyroid preparations, magnesium sulfate, sodium fluoride and the organic mercurials have variously been employed in preservation experiments with canine heart[18] and kidney[19] with minor success. The known preparations are either too nonspecific or too ineffective in action to be more than adjunctive measures. The action of these compounds is obscure and deserves further investigation.

In addition, membrane stabilizing agents have been used to delay or reduce the effects of hypoxia on cell membranes. Hypoxia is thought to produce cellular swelling due to plasma membrane damage with influx of sodium ions, and has been shown to disrupt intracellular organelles, particularly lysosomes, with release of acid hydrolases and consequent cell death. Agents such as chlorpromazine, the corticosteroids and chloroquine can experimentally stabilize various cell membrane systems.[20] These agents are again only of adjunctive interest.

2. SATISFACTION OF METABOLIC NEED

a. Ex Vivo Perfusion

Ex vivo organ perfusion implies the supply of nutrients to

tissue with artificially maintained circulation and would seem to possess the greatest potential for short and intermediate term storage periods because abundant oxygen, nutrients and hormones can be supplied with simultaneous removal of carbon dioxide and metabolic acids. A successful perfusion system for purposes of graft preservation would maintain an organ viable and totally functionally adequate.

Whole organ perfusion has been of interest for many years, emanating from the pioneer work of Alexis Carrel between 1914 and 1938. Carrel and Lindbergh established an elaborate perfusion apparatus which would sustain small excised organs for days and sometimes weeks with a variety of perfusates. Their techniques were inadequate for purposes of transplantation since no attempt at evaluation of graft viability was made aside from histologic appearance, and they were unable to preserve large parenchymal organs. In his classic monograph, The Culture of Organs[21] (1938), Carrel described the requirements for any successful perfusion system and these tenets remain valid today. He emphasized that the perfusate must be kept free of bacteria and embolic material; temperature, osmotic pressure and composition must be rigidly controlled and adequate oxygen and nutrients must be supplied.

Organ perfusion systems have been used extensively as basic research tools over the past 30 years. However, it was not until the past decade that perfusion has been considered as an experimental means of preserving organ viability and function. The requirement of normal or near normal function after a perfusion interval for purposes of transplantation has uncovered many physiologic problems. In normothermic blood perfusions of canine kidney,[22] liver,[23] spleen[24] and bowel,[25] a common difficulty has been increasing organ edema with

elevated vascular resistance and diminished venous outflow. This common syndrome in organ perfusion failure has been variously attributed to red cell, platelet, bacteria, fat, or lipoprotein[22,26,27] emboli with microvascular occlusion, or to release of vasoactive substances from tissue or circulating cells. Since whole blood appears relatively unaltered by the surfaces of modern plastics, there is reason to believe that oxygenators[22,27] are the principal culprits inducing erythrocyte and platelet aggregation, denaturation of plasma proteins, release of histamine and serotonin and possibly fat embolism. Belzer et al.[22] have found glass wool filtration to be an effective means of removing microemboli in isolated perfusion of dog kidneys with whole blood in a bubble oxygenator.

Most experience in isolated organ perfusion has been with isolated canine kidneys. Because of the difficulties in perfusing kidneys with whole blood, most investigators have turned to the use of acellular perfusates. Because acellular perfusates carry less oxygen than blood, hypothermia has been added to organ perfusion. Since the solubility of gases in aqueous solution is enhanced as temperature is decreased, plasma or other artificial perfusates can carry greater volumes of oxygen and carbon dioxide in solution at the same partial pressure. Hypothermia carries the added benefits of minimal damage to organs during transfer and reduced bacterial growth. Red cell perfusates work poorly at low temperatures since blood becomes more viscous and tends to sludge; hemoglobin binds oxygen very tightly, giving little up to the tissues and hence serves poorly as a buffer.[29]

With diluted blood and low flow under hypothermia the first 24 hour kidney perfusion was accomplished with immediate contralateral

nephrectomy after reimplantation.[30] Belzer et al.[31] have cryo-precipitated plasma to remove lipoproteins thought responsible for microvascular embolism and have successfully perfused canine kidneys under hypothermic conditions with this perfusate for 48 and 72 hours; after reimplantation, canine recipients have survived contralateral nephrectomy. Similarly, Humphries and coworkers[32] have perfused canine kidneys for 3 and even 5 days with adequate function on reimplantation.

When diluted or synthetic perfusates are used, control of colloid osmotic pressure becomes critical since saline and balanced electrolyte solutions rapidly lead to edema and rising vascular resistance in the perfused organ.[33] An additional problem with both cellular and acellular perfusates is maintenance of the normal composition. As the fluid is progressively altered by metabolism of the perfused organ, essential nutrients may disappear and toxic products may accumulate with subsequent death of the perfused organ. Many investigators have ignored this most essential aspect of extracorporeal circulation.

With all means of organ preservation it has become clear that a major problem is assessment of organ viability. Reimplantation or transplantation is the only available method but does not give the researcher immediate knowledge of the graft function or the nature of lesions induced during preservation. Perfusion itself may be an excellent method of assessment of functional viability as it allows simultaneous preservation and study.

b. Intermediate Host

The living animal is obviously the most perfect perfusion system available and thus temporary transplantation storage is one means of preserving an organ before its ultimate location. Ackermann

et al.[34] stored dog kidneys as allografts for 5 days and found them to function well after transplantation to a third dog. Angell and Shumway[35] have successfully perfused dog hearts via the cervical vessels of an intermediate host for 24 hours and found the same heart able to sustain life when transplanted orthotopically to a third dog. This means of preservation has little application at present, since the rapid rejection of organs between different species makes it inapplicable in the human situation.

B. PRESERVATION OF THE LUNG

1. LUNG PERFUSION

Perfusion of animal lungs, either isolated or in situ in the pleural cavity has been performed many times over the past fifty years. The lung was first used extensively in perfusion systems as a basic research tool in the elucidation of normal pulmonary physiology and is still used widely in this respect. From 1950 to 1956, the perfusion of animal lungs became more popular due to the development of extracorporeal circulation and investigators attempted to use isolated lungs as biologic oxygenators. In the last fifteen years, perfusion has been utilized experimentally as a means of infusing antitumor agents in high concentration into the isolated lung and as a possible method of oxygenating venous blood in temporary support of experimental respiratory failure. With widespread successful transplantation of canine lungs since 1962, experimental perfusion of animal lungs has acquired current importance as a means of preservation.

a. Perfusion Systems in Research Pulmonary Physiology.

Although several investigators[36,37] worked with isolated lung

and heart models before the turn of the century, Verney and Starling[38] in 1925 first implemented isolated heart-lung preparations as physiologic oxygenators for kidney perfusion experiments. These researchers were not concerned with the factors implicated in maintaining prolonged pulmonary function but Hemingway,[39] also studying the function of isolated kidneys (1931), noted that his lung oxygenator would eliminate vasoconstrictor activity present in defibrinated blood as well as oxygenate the perfusate. In 1937 Alcock[40] perfused dogs' lungs in situ with negative pressure ventilation and found that with intact vagal and sympathetic innervation intravenous histamine would diminish tidal volume.

Daly[41] in 1938 first described a method for perfusion of isolated lungs of the guinea pig, dog and monkey with negative pressure ventilation and did much of the early work on the effects of histamine and serotonin on the bronchial tree. The studies performed by this investigator established much of the methodology since used in lung research. Duke[42] extended the work of Daly and others and in experiments with isolated perfused canine lungs, outlined the hypoxic pressor response. She found that inhalation of inspired gas of oxygen content less than 15% or of 5-10% CO_2 produced an increase in pulmonary artery pressure that was not abolished by atropine. In addition, perfusion of isolated lungs with partially deoxygenated blood did not give rise to the pressor response identified with ventilating gases. Duke's preparations incorporated both positive and negative pressure ventilation in separate experiments; the perfusate consisted of diluted defibrinated blood. Microscopic edema, congestion and atelactasis

accompanied all the two to three hour perfusions.

Nissel[43] in 1948 perfused isolated cats' lungs at normothermia with constant blood flow and also identified the pressor response to alveolar hypoxia. He ventilated lungs with both positive and negative pressure ventilation and concluded that negative pressure ventilation produced lungs that were grossly edematous after four hours perfusion while positive pressure ventilation gave rise to only slight edema after six or seven hours perfusion.

In the past 20 years, studies too numerous to mention have implemented perfusion systems to elucidate sophisticated pulmonary physiology. Knowledge of the behavior of the pulmonary circulation and the effects of inflation on its hemodynamics has increased rapidly since 1951 when Riley and Cournand[44] published their classic papers on the pulmonary ventilation-perfusion relationships. In 1960 Thomas[45] ventilated an isolated lung with negative pressure to establish that the pulmonary vascular resistance was lowest at approximately half maximal lung volume and increased on either further inflation or deflation. Banister and Torrance in 1961 published a classic paper[46] in which an isolated lung perfused with steady flow plasma dextran mixture was used to determine the influence of tracheal pressure on the pressure characteristics of the lung. They found that pulmonary arterial pressure was affected by venous pressure only when the venous pressure approached tracheal pressure and that under steady states of flow and inflation, increases in tracheal pressure reduce the conductance of the pulmonary blood vessels. They explained that the alveolar capillaries behave like sluices or Starling resistors. Permutt et al.[47] confirmed the work of Banister and Torrance and

described the pulmonary circulation in terms of a vascular waterfall. In 1964 West[48] extended this line of inquiry with isolated perfused dog lungs and emphasized the normal distribution of blood flow in the lung, gravitational effects and relation to vascular and alveolar pressures.

b. Lung Perfusion in Extracorporeal Circulation

Studies in the perfused lung were given impetus in the early 1950's with the initial work in clinical cardiopulmonary bypass. Homologous and heterologous lungs were evaluated extensively as possible biologic oxygenators in extracorporeal circulation. In 1951 Potts[49] oxygenated the blood of nonventilated anesthetized dogs for periods up to six hours by perfusing ventilated homologous dog lungs with venous blood and subsequent return of arterial blood to the animal. Wesolowski et al.[50] attempted to perfuse dog lungs with human blood but severe congestion and pulmonary edema occurred after just twenty minutes of perfusion. He found, however, that the same system would oxygenate dog lungs perfused with homologous blood satisfactorily enough to permit survival of five dogs that underwent two hour cardio-pulmonary bypass.

Crisp, Campbell and Brown[51] courageously used dog lungs as oxygenators in human extracorporeal circulation in 1955. Isolated canine lungs were ventilated with positive pressure and 100% oxygen for periods ranging from 30 to 150 minutes without gross edema. In spite of low flows (350 to 1000 cc/minute) for both lungs, pulmonary edema and congestion was evident microscopically and became more severe with higher flow rates. These workers felt that edema was minimized with an initial dextran flush of the lungs, pulmonary veins open to gravity drainage, depulsation of pulmonary arterial

flow and avoidance of ventilatory obstruction. Mustard and Chute[52] similarly used primate lungs as oxygenators in clinical extracorporeal circulation and noted similar successes. Cohen and Lillehei[53] experimented with autologous canine lungs as oxygenators and iterated the adequate gas exchange possible, but inevitable early appearance of pulmonary edema even at low flows. Development of the more convenient and reliable disc and bubble oxygenators discouraged interest in animal lung oxygenators after 1956.

c. Lung Perfusion in the Chemotherapy of Tumors

Between 1959 and 1962 interest focussed on isolated perfusion of the lung as a means of delivering high effective concentrations of antitumor agents. Pierpont and Blades[54] cannulated the pulmonary artery and vein of canine lungs in situ and infused nitrogen mustard mixtures in blood. In later experiments Pierpont[55] was able to perfuse the in situ lung for periods as long as four hours and even with retrograde perfusion through the pulmonary veins without gross edema. The ventilation of the perfused lungs in these instances was accomplished with the use of a whole body negative pressure respirator.

d. Lung Perfusion in Experimental Respiratory Failure

Moore and Eiseman began in 1965 to perfuse animal lungs as possible assisting oxygenators in reversible pulmonary disease. The perfusion system incorporated heparinized blood as a perfusate, positive pressure ventilation and gravity venous drainage. In 1967 reporting on 137 pig lungs perfused with human blood, Eiseman[56] found that the animal lungs were efficient oxygenators of human blood but described a progressive rise in vascular resistance over the course of a 4 to 6

hour perfusion, partially diminished by isoproterenol. The flow rates in this series were not specifically mentioned but were kept low in relation to the normal flow through a single pig lung in order to minimize edema. This prompted Eiseman to comment that "until a means can be found to increase blood flow to at least 750 ml/minute the preparation will be unsuitable for clinical pulmonary assistance". As an interesting minor facet of this work, Eiseman and coworkers[57] found that elevated histamine or serotonin concentrations in their lung perfusate elevated pulmonary vascular resistance, tracheal resistance and diminished blood flow. Furthermore, the isolated lung could metabolize serotonin but not histamine.[57]

Bryant and colleagues[58] used porcine lungs as experimental oxygenators for human blood in a perfusion system that similarly incorporated intermittent positive pressure ventilation and gravity venous drainage. Prior to perfusion the lungs were flushed with two litres of lactated Ringer's solution and this in itself caused a mean weight gain of 75 grams. In autologous perfusions, gross edema was noted between the second and third hours of perfusion and the flow had diminished by approximately 50%. Perfusion with human blood resulted in early edema and rapid deterioration of pulmonary function. A major problem in this series was that of high pulmonary vascular resistance with human blood perfusion and routine flows of only 500 cc/minute were obtained, less than half of that possible with autologous pig blood. The average course of perfusion was 2.1 hours with progressive edema and atelectasis noted during the final hour of most experiments. A trial of negative pressure ventilation resulted

in lower pulmonary vascular resistance, but appeared to hasten the development of edema. The use of isoproterenol resulted in somewhat better flow rates with less edema.

Ratliff[59] commented that the lesions found in lung perfusion and other systems have implicated as causes homologous blood, heart worms, heparin, etc., but that the raw blood gas interface of oxygenators still appears to be the major cause of lethal pulmonary changes. In experimental respiratory support, therefore, five apneic dogs were supported for as long as three hours by partial venovenous prepulmonary perfusion with an homologous lung oxygenator. Although longer perfusions were not achieved the author concluded that the key to functional integrity in the perfused lung was the maintenance of a low left atrial outflow pressure.

e. Lung Perfusion in Experimental Preservation Studies

Efforts to perfuse isolated lungs for purposes of short term preservation are relatively recent. In 1966 Veith, at the Montefiore Hospital in New York, developed a lung perfusion system[60] with this in mind. Essential features of his perfusion system included positive pressure ventilation, gravity venous drainage, and the presence of a bubble oxygenating unit. Attempting to find an optimal perfusate he evaluated differing anticoagulants,[61] fresh and stored blood and temperature and found least damage to isolated perfused canine lungs occurring with ACD anticoagulated fresh blood at 28°C. The same preparation was used to investigate the pulmonary changes common to isolated lung perfusion, venovenous bypass and total cardiopulmonary

bypass.[62] Although 3 hour perfusions were attempted, 13 of 20 experiments had to be terminated in less than 3 hours due to froth pouring from the trachea. Microscopic study of lungs subjected to perfusion, venovenous bypass and total cardiopulmonary bypass revealed alveolar edema and periarterial hemorrhage in all instances, from which it was inferred that the common interaction of blood with an extracorporeal gas exchanger was responsible for the observed alterations.

Awad[63] perfused dog lungs in situ under similar physical conditions, but found an 85% mortality after restoration of normal flow. He felt that the atelectasis and hemorrhage in perfused lungs was due to several factors: loss of surfactant, non-physiologic pressure in pulmonary vessels, sludging, impeded venous return and denaturation of plasma proteins. In further work, Awad[64,65] found that perfusions longer than 3 hours were possible with the addition of low molecular weight dextran to the perfusate and that interruption of the bronchial circulation and addition of cortisone to the perfusate were of some beneficial effect.

Brownlee and colleagues[66] perfused left lower pulmonary lobes with unfiltered plasma at 15°C and found that both negative and positive pressure ventilation resulted in marked edema and approximately 100% weight gain within 3 hours of perfusion. As well the perfused lobes released histamine into the perfusion system. Lunde[67] had earlier documented the intolerance of the canine lung to perfusion with unfiltered plasma.

Otto[68] perfused canine lungs at 15°C hypothermia with a perfusate of blood diluted with Rheomacrodex. He found the lungs very

intolerant to hypothermic perfusion and, testing the perfused lungs by allografting, had only one chronic survival after 5.5 hours of perfusion. In this workers experience ventilation without perfusion under hypothermia resulted in failure. The effect of hypothermia on lung surfactant was investigated by Miller and Kuenzig[69] who found no significant decrease in surface activity in either normothermia or hypothermic groups immediately after perfusion. Miller suggested that increased left atrial pressure or direct pulmonary arterial perfusion were causative factors in perfusion lung damage.

Homatas and Bryant[70] implemented a perfusion system to investigate the tolerance of cadaver lungs to ischemia and found that the maximum period following death during which a canine lung will maintain its effectiveness for subsequent in vitro gas exchange is at least two hours if the lung is left unventilated within the cadaver. If the lungs are artificially respired within the cadaver, this period of preservation may be as long as six hours. During the perfusion studies on anoxic lungs these authors outlined factors which, in their experience, had produced more successful perfusions:

(1) minimal manipulation of the lung,

(2) unobstructed venous drainage at -5 cm HOH,

(3) intermittent sighing during ventilation,

(4) positive endotracheal pressure of 7 to 8 cm HOH,

(5) avoidance of expiratory resistance,

(6) pulmonary artery pressure less than 20 cm HOH and flow less than 2 litres/minute for both lungs,

(7) turning the donor to minimize dependent pooling in the lung,

(8) minimal lung washout with electrolyte solutions

(9) physiologic perfusate

Deoxygenation of the heparinized blood perfusate was accomplished with nitrogen and carbon dioxide in a disc oxygenator.

Lung perfusion technology was significantly advanced by Fisk, Dritsas and Couves. A sophisticated organ perfusion apparatus was used by Fisk and coworkers[71,72] to successfully perfuse canine lungs, brains, hearts and stomachs. For left lower pulmonary lobe perfusions, Fisk utilized the donor animal as a deoxygenator for arterialized blood and ventilation of the lobe was accomplished with intermittent negative pressures established to produce a tidal volume of 5 cc/donor kgm. Replicating the normal flow rates and with positive venous pressures (mean 7 mm Hg), pulmonary lobes were routinely perfused for five or six hours without gross edema and with functional and morphologic integrity. Both Fisk[72] and Dritsas[73] emphasized the importance of positive venous pressures in the perfused lung; Couves[74] and associates had earlier indicated the general desirability of maintained venous pressure in the systemic microcirculation.

Clarke[75] developed a technic of perfusing the isolated lung for preservation in which flow was accomplished by means of successive respiratory excursions without a perfusion pump. Canine lungs were subjected to positive and negative pressure inflation in a sealed chamber and were perfused with blood or blood mixed with low molecular weight dextran at either 23 or 36°C. In all experiments, gross histologic changes and pulmonary edema were evident within 4 hours and only marginally longer perfusions were obtained than in a control

series in which a roller pump was used. In another series[76] Clarke evaluated the use of pulsatile and nonpulsatile flow on lungs and found that there was no demonstrable impairment of the function of the lung as an oxygenator during nonpulsatile perfusion, despite the development of some alveolar wall thickening on microscopic examination. The main difference with nonpulsatile as compared to pulsatile flow in Clarke's experience was an increased pulmonary vascular resistance which was proportionately greater at higher flow rates. Maloney and associates[77] also compared pulsatile and nonpulsatile flow during isolated lung perfusion and found that the pattern of blood flow measured with Xe 133 was altered during pulsatile perfusion so that more blood passed through the upper zones of lung. He suggested that the transmission of the pulmonary artery pressure pulse by the blood vessels of the lung is frequency dependent and that this alters distribution of blood flow. It was concluded that the lung passed well only the low frequency components of the applied input and that in a vertical lung only about 30% of the mean to peak pressure pulse would be transmitted down to the small vessels and therefore affect distribution of blood flow.

f. Pathology of the Perfused Lung

The gross and microscopic pathology of perfused lungs has been known for many years and the first account was given as early as 1944 by Trowell.[78] Trowell implemented the perfusion technique of Daly and perfused canine lungs with heparinized blood for periods between 3½ and 7 hours. In careful study of formalin fixed, hematoxylin and eosin stained specimens after perfusion, the main findings were:

(1) edema with alveolar exudate, distension of periarterial lymphatics and edema of arterial walls,

(2) periarterial and peribronchial hemorrhage,

(3) collections of polymorphonuclear leucocytes in the small pulmonary blood vessels,

(4) dilatation of bronchi and bronchioles,

(5) vascular congestion of bronchial walls.

Numerous investigators[61,62,79] have since confirmed the presence of these lesions and have emphasized that gross edema, congestion and hemorrhagic atelectasis in the failing perfused lung are accompanied microscopically by interalveolar edema and congestion, alveolar exudate, microalveolar collapse, extravasation of red cells with breakdown of the alveolar membrane and periarterial hemorrhage. These findings have variously been reported with almost equal frequency in the shock lung syndrome,[80] respirator lung,[81] oxygen toxicity,[82] various embolic states,[83] septicemia[84] and the lung after prolonged cardiopulmonary bypass.[62] Many possible etiologic agents have been implicated: intravascular coagulation, loss of surfactant, peri-arteriolar and periarterial spasm with subsequent hemorrhage, direct effect of hypoxia on vessels and alveolar walls, blood platelet, fibrin, bacterial and lipoprotein emboli. Rather than any single mechanism satisfactorily explaining these events, it may be that the lung reacts in a limited fashion to a wide variety of specific noxious stimuli.

2. PRESERVATION OF THE LUNG WITHOUT PERFUSION

The tolerance of the canine lung to ischemia was first investigated in 1952 by Blades and coworkers,[85] who showed that in situ occlusion of the pulmonary circulation through canine lungs was well tolerated for periods ranging from 30 to 360 minutes. Long and colleagues[86] found however, that normothermic occlusion of the pulmonary artery for three hours in situ resulted in diminished compliance and pulmonary surface activity several days following. Other investigators found that temporary hilar occlusion in sheep lungs for four hours would regularly produce pulmonary edema. Consequently Hardy[88] in 1963 investigated hypothermia as a possible means of protecting the ischemic lung prior to reimplantation and found that storage of canine lungs filled with Tyrode's solution at $4^{o}C$ occasionally permitted survival of reimplanted lungs after 24 hours storage. Lung function was inadequately assessed after reimplantation.

Blumenstock et al.[89] similarly replaced the pulmonary blood volume in left canine lungs with high molecular weight dextran, serum mixed with dextran, or serum buffered to remove carbon dioxide. The lungs were placed in plastic bags and immersed in ice water for periods from 18 to 28 hours with periodic positive pressure inflation; after the storage interval the lungs were allografted. Only lungs treated with buffered serum permitted graft survival (4 to 6 animals) and were histologically comparable to nonstored controls. Immunosuppression was carried out with methotrexate and no pulmonary function studies on the preserved lobes were reported.

In 1964 Manax and Lillehei[6] aroused great interest in organ

preservation when they found that canine kidneys stored at 2°C and under 3 Atmospheres absolute pressure with hyperbaric oxygen were able to sustain life after 24 hours preservation and delayed contralateral nephrectomy. They extended their techniques to other organs including canine hearts, bowel segments, and lungs, and commented that "in 4 dogs successful lung storage has been accomplished for 48 hours at 2°C and 3 OHP", but did not elaborate on what means, if any, were used to evaluate these storage procedures.[7] In a later publication (1965) the same investigators stored lungs at 3 Atmospheres absolute hyperbaroxia and 2°C for 24 hours and evaluated the stored lungs as allografts in other dogs. Preservation was considered succesful if the recipient animal survived allografting and had intact anastomoses at sacrifice 2 to 10 days post surgery. Oxygen at pressures greater than 3 Atmospheres absolute pressure (30 psi) was found to be toxic to lung tissue.

In similar fashion Blumenstock et al.[91] stored canine lungs at 2 Atmospheres hyperbaria and 4°C and found that 8 of 22 lungs were successfully preserved in allografted dogs immunosuppressed with methotrexate. Criteria of success was normal microscopic appearance of the pulmonary architecture 6 to 72 days following transplantation. Garzon and coworkers[92] recognized that allografting of the preserved organ interferes with accurate evaluation of preservation techniques and accordingly, preserved lungs for 24 hours at 4°C and 2 Atmospheres pressure, but reimplanted the preserved lungs after the storage interval. Eight of 20 animals survived this regimen and of these five had adequate function on delayed bronchospirometry. Again the

immediate post preservation function of stored lungs was not assessed.

Ross and Alves[93] attempted to combine hyperbaric oxygen, hypothermia and perfusion, but found no survivors in animals reimplanted after one hour exposure to 3 Atmospheres oxygen, 4°C and perfusion with or without ventilation. In contrast 20 of 27 animals with lungs exposed to hypothermia and hyperbaric oxygen survived reimplantation.

Theodorides[94] evaluated metabolic inhibition with respect to lung preservation and instilled, but did not circulate, fluid into the pulmonary artery with subsequent reimplantation. They found that a solution of magnesium, glucose and low molecular weight dextran maintained satisfactory viability of the lung in situ at normothermia for over 4 hours and at 4°C for over eight hours. Functional studies three to four weeks after grafting were used to evaluate the efficacy of preservation.

C. OBJECTIVES

From the foregoing, it is evident that there are two main methods of preserving pulmonary tissue - by perfusion and by non-perfusion preservation with hypothermia and hyperbaric oxygen. The fund of information available regarding lung preservation attempts is deficient in several respects:

(1) While there is considerable evidence to indicate that canine lungs preserved for 24 hours under hyperbaric-hypothermic conditions are viable, there is no information concerning the immediate post preservation function of such lungs. For transplantation purposes, satisfactory preservation must include both viability and totally adequate function immediately after termination of the storage interval. This implies that any preservation method must entail a means of

evaluating function if it is to be significant.

(2) Perfusion preservation of the isolated lung has been singularly difficult compared with other organs. Where perfusion of many systemic organs with hypothermic acellular perfusates has been notably successful for periods of 24 hours or more, this has not been true with the lung. The most successful lung perfusion experiments have occurred with autologous blood perfusate at normothermia, and even with simulation of normal pulmonary hemodynamics, these can routinely maintain adequate pulmonary morphology and function for only 5 or 6 hours.

(3) Investigators have realized that perfusion of an organ with abundant nutrient materials would seemingly provide the most excellent functional support. In spite of this, workers intending to preserve the isolated canine lung have failed to consider the changing biochemical environment of a closed perfusion system. Depletion of substrates, accumulation of metabolic acids and release of toxic or vasoactive materials from damaged tissue would all seem eminently possible.

(4) The lung is unique in that ventilation can profoundly affect the circulation through a low pressure bed. Most investigators of the isolated perfused lung utilize positive-pressure ventilation, probably because of its technical facility. In spite of this deviation from the normal physiology, many workers feel that positive pressure ventilation is necessary to prevent pulmonary edema in the isolated lung. The different hemodynamic and functional responses to positive and negative pressure ventilation remain to be evaluated in terms of

isolated lung perfusion.

The problems outlined above suggested four major questions in experimental preservation of the isolated canine lung that could profitably be explored with the use of an organ perfusion apparatus:

1. How efficient is the functional preservation of the lung exposed to hypothermia and hyperbaric oxygen?
2. Can more rigid simulation of the normal intrathoracic hemodynamic and metabolic environment yield longer functional preservation of the isolated canine lung than heretofore reported?
3. What are the biochemical changes that must be anticipated and modified in isolated unsupported lung perfusion?
4. How are the hemodynamics and function of the isolated perfused lung altered by positive pressure ventilation?

These questions are considered in detail in Chapters III-VI.

CHAPTER II

THE PERFUSION APPARATUS AND TECHNIQUE OF ISOLATED LEFT LOWER PULMONARY LOBE PERFUSION

1. THE UNIVERSITY OF ALBERTA ORGAN PERFUSION APPARATUS

The apparatus used in this study to perfuse isolated pulmonary lobes was constructed at the University of Alberta. The recommendations of a series of research workers interested in organ perfusion technology, were assimilated and modified by Dr. R.L. Fisk with final assembly of a versatile perfusion unit.

The organ chamber is the principal component of this system and has an internal capacity of 3.38 cubic feet. The walls are constructed of two 1/8" thick stainless steel plates, separated by 5/8" to form a water jacket for chamber temperature control. The front of the chamber functions as a drawer assembly and slides on bearings attached to the outside of the chamber. A handlock permits rapid sealing or entry to the chamber. The platform which supports the organ is attached at the front of the chamber and in turn rests on a weight transducer by means of which constant organ weight is determined. Interchangeable fittings in the front panel window connect the perfusion circuit with the cannulated pulmonary artery, vein and bronchus of the pulmonary lobe. Cannulas are designed to sense lateral pressure in the vessel beyond the cannula tip. A special drainage cannula allows gravity drainage of fluid collections within the chamber.

The perfused organ is easily visualized through five removable lucite windows of 10" diameter. Humidification of the chamber is provided through a saline pool in the bottom of the chamber. This allows a humidity of 100% to be attained in the chamber two minutes following closure. Water which

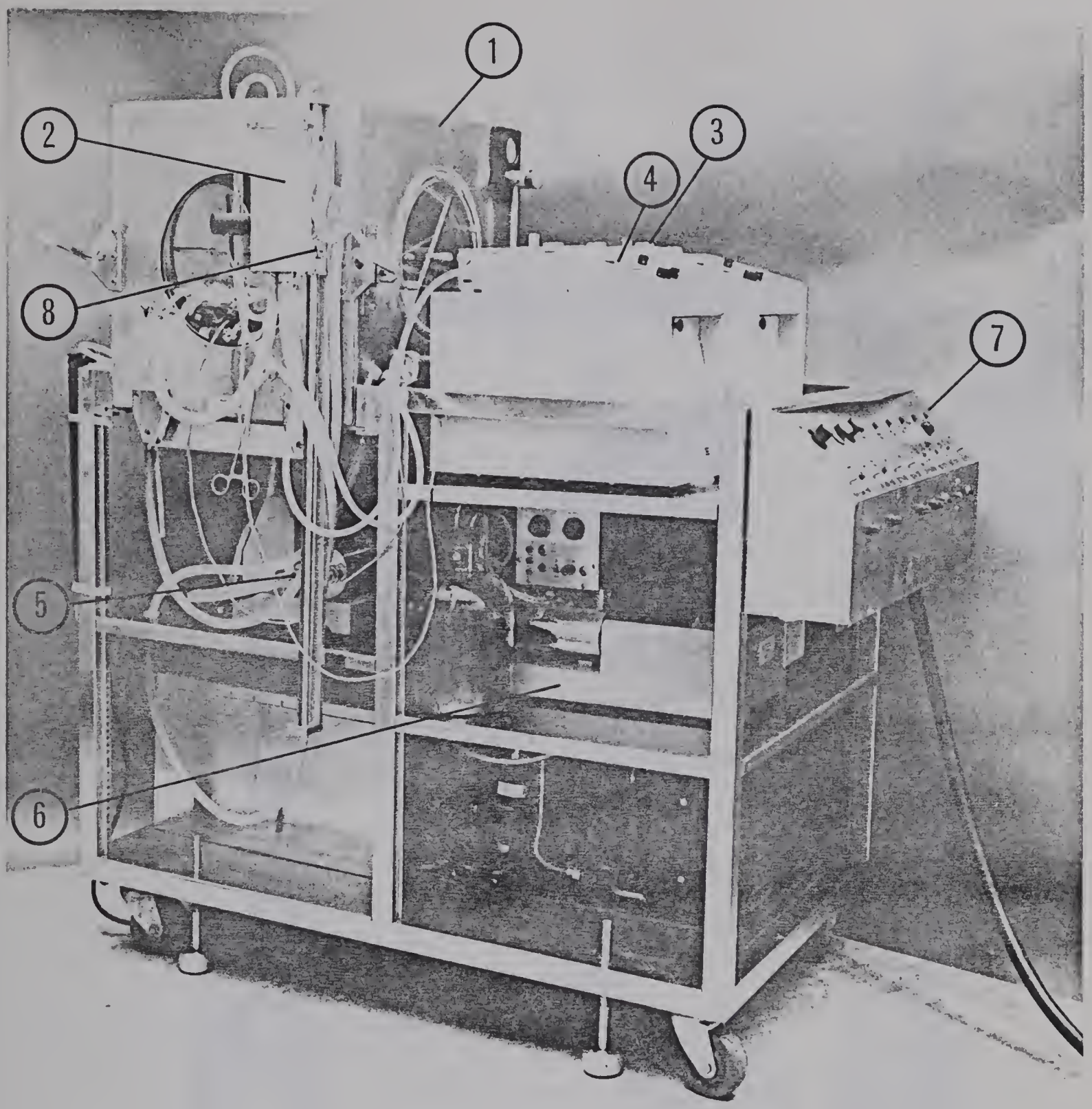


Fig. 1. Apparatus designed for study of the isolated perfused canine lung.

1 - perfusion chamber
2 - venous reservoir
3 - arterial pump
4 - venous pump
5 - water bath
6 - respirator pump
7 - electronic control panel
8 - photocell for venous pump control

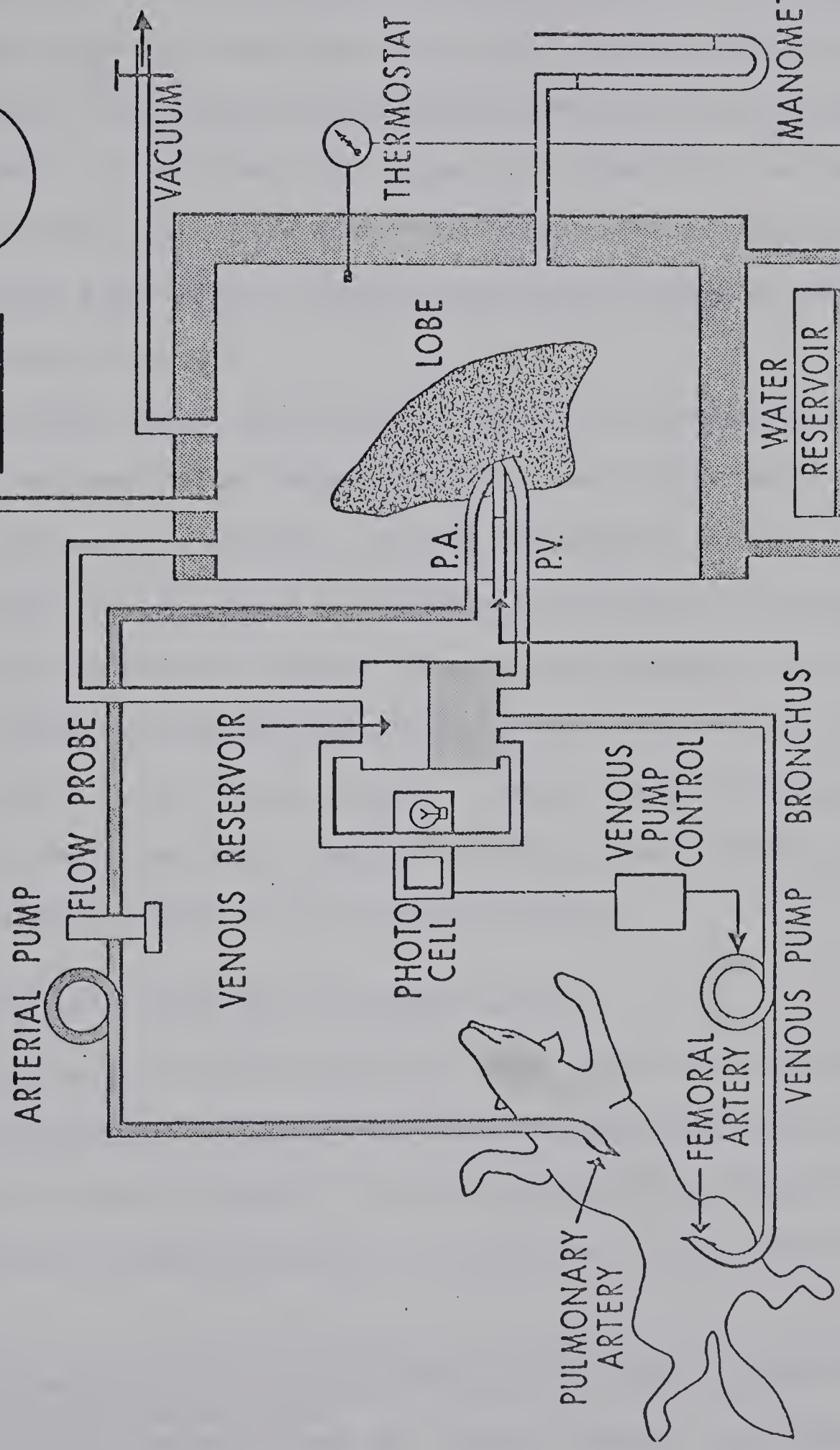


Fig. 2. Schematic representation of lung perfusion circuit.

accumulates on the interior of the lucite windows is cleared by externally controlled wiper blades.

Ports on the back wall conduct transducer cables into the chamber and through a separate connection, a specially designed Harvard Respirator pump* controls intermittent manometrically controlled pressure changes in the chamber. A water bath and heater are located below the chamber, and are thermostat controlled within two degrees of a selected temperature. From the water bath, water is pumped through water jackets on the chamber and main venous reservoir.

An occlusive roller pump¶ delivers blood to the isolated lobe. The arterial flow rates can be varied manually or can be governed to deliver that flow necessary to maintain a preselected arterial pressure. The venous outflow from the organ is conducted to an adjustable reservoir located near the chamber entrance. Photoelectric sensing of the blood level in the venous reservoir permits maintenance of a constant venous pressure with return of excess blood to a support animal or deoxygenator system at a determined rate. The extracorporeal blood flow is conducted through polyvinyl tubing of 3/8" internal diameter.

2. TECHNIQUE OF SURGERY AND THE SUPPORT ANIMAL

A large mongrel dog is anesthetized with 30 mgm/kgm intravenous sodium pentobarbital, intubated with a cuffed endotracheal tube and placed in the right decubitus position. Positive pressure ventilation§ with oxygen-air mix is instituted prior to thoracotomy through a left fifth

---

* Harvard Respirator Pump Special Product #1170, Harvard Apparatus Co.Inc., Dover, Massachusetts, U.S.A.

¶ Sarns Low-Flow Perfusion Pump (No. 5M6050) Travenol Laboratories Ltd., Alliston, Ontario.

§ Bird Mark VII Respirator, Bird Corporation, Palm Springs, California.

interspace incision. Initial upper lobectomy is performed with immediate fixation in 10% formalin for control lung histology. The left lower lobe is mobilized with care taken to preserve long sections of pulmonary artery, bronchus and pulmonary vein including a small cuff of left atrium. The left lower lobe is then excised and weighed. In a standard perfusion the lobe is placed on the tray in the perfusion chamber and pulmonary artery, bronchus and vein with atrial cuff are cannulated in sequence.

The donor animal can be utilized as a convenient means of deoxygenating the arterialized blood from the perfused lobe. If this is desired the proximal left artery in the donor animal and a femoral artery are cannulated and connected to the perfusion circuit as shown in Fig.2. Alternately arterialized blood may be returned to a bubble oxygenating unit through which controlled flow of carbon dioxide and nitrogen maintain correct venous gas concentrations. If a support animal is maintained in the perfusion circuit 30 mgm increments of pentobarbital and buffered Ringer's solution are admininstered as required. The circuit is heparinized with an initial dose of 3 mgm/donor kgm heparin and an additional 1 mgm/kgm every hour thereafter.

3. MEASUREMENTS

The venous and arterial pressures of isolated perfused lobes are measured with catheters to transmit the lateral pressure in the pulmonary vessels beyond the ends of the flow cannulas to strain gauge transducers* recording continuously on a polygraph recorder.¶ From arteriovenous

---

* Statham P23 Series Pressure Transducers, Bionetics Ltd., Montreal, P.Q.
¶ Offner Type R Dynograph Recorder, Beckman Instruments Inc., Palo Alto, California, U.S.A.

pressure differences and known flow rate through the lobe, pulmonary vascular resistance is calculated. Organ weight is similarly continuously monitored with a platform transducer and, if applicable, donor arterial pressures are recorded. Lobe tidal volumes are measured by spirometry and with chamber pressure excursions determine compliance. Lobe and support arterial and venous pH, pO_2 and pCO_2 are measured* in heparinized blood samples as often as desired. Following perfusion, the left lower lobe is weighed and fixed in formalin for subsequent histologic examination.

---

* Radiometer Type PHA 927 Physiologic Blood Gas Monitor, Bach Simpson Ltd., London, Ontario.

CHAPTER III

EX VIVO EVALUATION OF STORED LUNGS

A number of investigators[90-92,95,96] have stored canine lungs for periods of 24 hours or longer with hypothermia and hyperbaria. Garzon et al.[95,96] stored six left lower lobes for 24 hours at 2 Atmospheres hyperbaria with oxygen and at 4°C. The reimplanted lobes were evaluated in situ more than three weeks postoperatively and apparently functioned similar to lobes which were immediately reimplanted. Other investigators[90,91] have attempted to assess the adequacy of their preservation methods by the survival of allograft recipients which were allowed to retain normal contralateral lungs. In the case of the lung or heart, the preserved organ must be capable of almost total normal function immediately following transplantation. No attempts to evaluate the function of lungs immediately following storage have been reported.

It was decided to preserve canine lobes by the method described by Garzon et al.[95,96] and to evaluate their function immediately following storage using a method of isolated perfusion. The objectives in this exercise were to evaluate the adequacy of a current method of lung preservation and to assess the usefulness of a lung perfusion unit in this regard.

Methods and Materials

Left lower pulmonary lobes from 18 healthy 16 to 32 kgm mongrel dogs were used in this study; nine served as controls and nine lobes were stored. The method of lobe excision was described in Chapter II. Following excision, the lobes were flushed ex situ with cold (4°C) buffered heparinized Ringer's lactate solution with concomitant ventilation. In the nine control lobes, when blood had

been flushed from the vasculature of the lobe, the hilar structures were connected to cannulas in the perfusion chamber and perfusion was instituted. Following flushing, the nine test lobes were stored in a hyperbaric bell under 2 Atmospheres absolute pressure (15 psi), immersed in buffered Tyrode's solution and oxygen, in a cold room controlled at $4^{o}C$. After the 24 hours storage interval, pressure in the hyperbaric chamber was released over a five minute period and the lobe was then perfused in the same manner as the controls.

The organ perfusion apparatus was identical to that illustrated in Figs. 1 and 2. A homologous support animal was employed in the circuit as a convenient deoxygenator. Once ventilation of each lobe was begun, venous blood flow was initiated gradually from the proximal segment of the left pulmonary artery of the support animal to the distal arterial segment of the lobe in the perfusion chamber. Over 15 minutes the blood flow was gradually increased to a constant 15 cc/donor kgm/minute.[97] Arterialized blood was conducted from the cannulated left atrium and pulmonary vein to the venous reservoir. A second occlussive roller pump returned this oxygenated blood to the femoral artery of the support animal. The return pump rate was automatically controlled by photoelectric sensing of the blood level in the venous reservoir and a constant mean venous pressure of 2 mm Hg was maintained. Ventilating pressures were adjusted to produce 4-5 cm HOH negative pressure fluctuations per breath and the respiratory rate was arbitrarily maintained at 10-12 per minute with periodic hyperinflations. All lobes were ventilated with room air.

Pulmonary arterial and venous pressures, serial arterial,

venous and support blood gases were recorded every fifteen minutes for the first hour of perfusion and every thirty minutes thereafter. Simultaneous lobe tidal volumes and negative ventilation pressures were recorded. The weight of each lobe was continuously monitored during perfusion. Representative sections of all stored and perfused lobes were fixed in formalin for histologic study.

Results*¶

The nine control lobes functioned well for at least four and, in most cases, five hours of perfusion. During this period they maintained consistently adequate oxygenation and carbon dioxide release across the perfused lobe. Compliance was well maintained. Over the four hour perfusion period (Table I) pulmonary function in the control lobes declined slowly and this was paralleled by gradual weight gain. One of the control experiments was terminated between four and five hours of perfusion, but the remaining eight lobes were electively discontinued at this time.

In the group of nine left lower lobes which were subjected to 24 hours storage, the level of function was markedly different (Table II). Although initial gas transfer and compliance were adequate, these and all other parameters deteriorated after only 15 to 45 minutes of perfusion (Figs. 3-5). Thereafter weight gain rapidly progressed, tidal volumes fell precipitously and gas transfer across the lobe was not measurable. Perfusion was discontinued in eight of the lobes after 60

---

* A sample dynograph tracing is presented in Appendix I.

¶ Sample venoarterial oxygen gradients are presented in Appendix II.

TABLE 1 - AVERAGE PULMONARY HEMODYNAMICS AND FUNCTION OF 9 CONTROL LEFT PULMONARY LOBES HOMOLOGOUSLY PERFUSED FOR 4 HOURS

Time of Perfusion (minutes)	Pulmonary Arterial Pressure (mm. Hg)	Pulmonary Vascular Resistance (dynes sec. $cm.^{-5}$)	Lobe Compliance (cc./cm. HOH/ lobe gm.) x100	(A-V) CO_2 Difference (mm. Hg)	(V-A) O_2 Difference (mm. Hg)
0	9.3	6150	30.7	25.7	49.9
15	12.6	3030	35.6	16.7	35.9
30	12.6	2930	30.4	15.1	27.4
45	12.6	2960	30.4	9.4	31.3
60	13.0	2960	31.1	10.8	33.0
90	12.2	2680	30.4	9.8	33.7
120	12.9	3160	29.0	8.7	32.1
150	12.2	3040	25.1	13.8	28.6
180	11.6	2360	29.0	14.3	31.8
210	12.8	2800	23.1	13.3	32.2
240	12.6	2930	22.0	10.6	27.9

TABLE II - AVERAGE PULMONARY HEMODYNAMICS AND FUNCTION OF NINE LEFT LOWER PULMONARY LOBES STORED FOR 24 HOURS WITH SUBSEQUENT HOMOLOGOUS PERFUSION

Time of Perfusion (minutes)	Pulmonary Arterial Pressure (mm. Hg)	Pulmonary Vascular Resistance (dynes sec. cm.$^{-5}$)	Lobe Compliance (cc./cm. HOH/ lobe g.)x 100	(A-V) CO_2 Difference (mm. Hg)	(V-A) O_2 Difference (mm. Hg)
0	9.7	4860	13.2	18.1	44.4
15	14.4	2730	12.0	7.6	13.9
30	15.5	2840	8.9	5.3	4.4
45	16.7	3090	8.8	6.2	6.2
60	19.3	3640	5.5	4.6	5.9

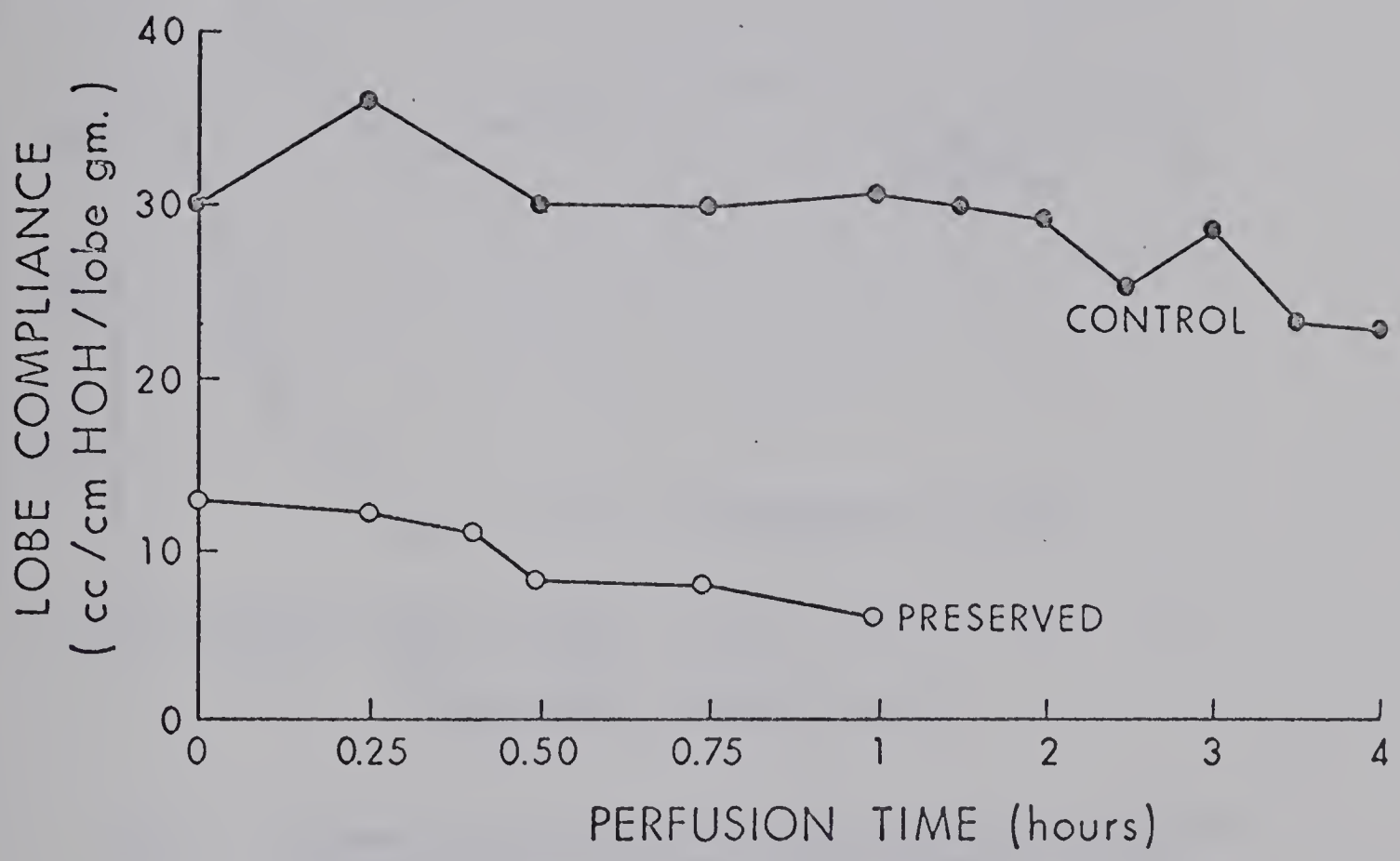


Fig. 3. Pulmonary compliance during perfusion of control and 24 hour stored lobes.

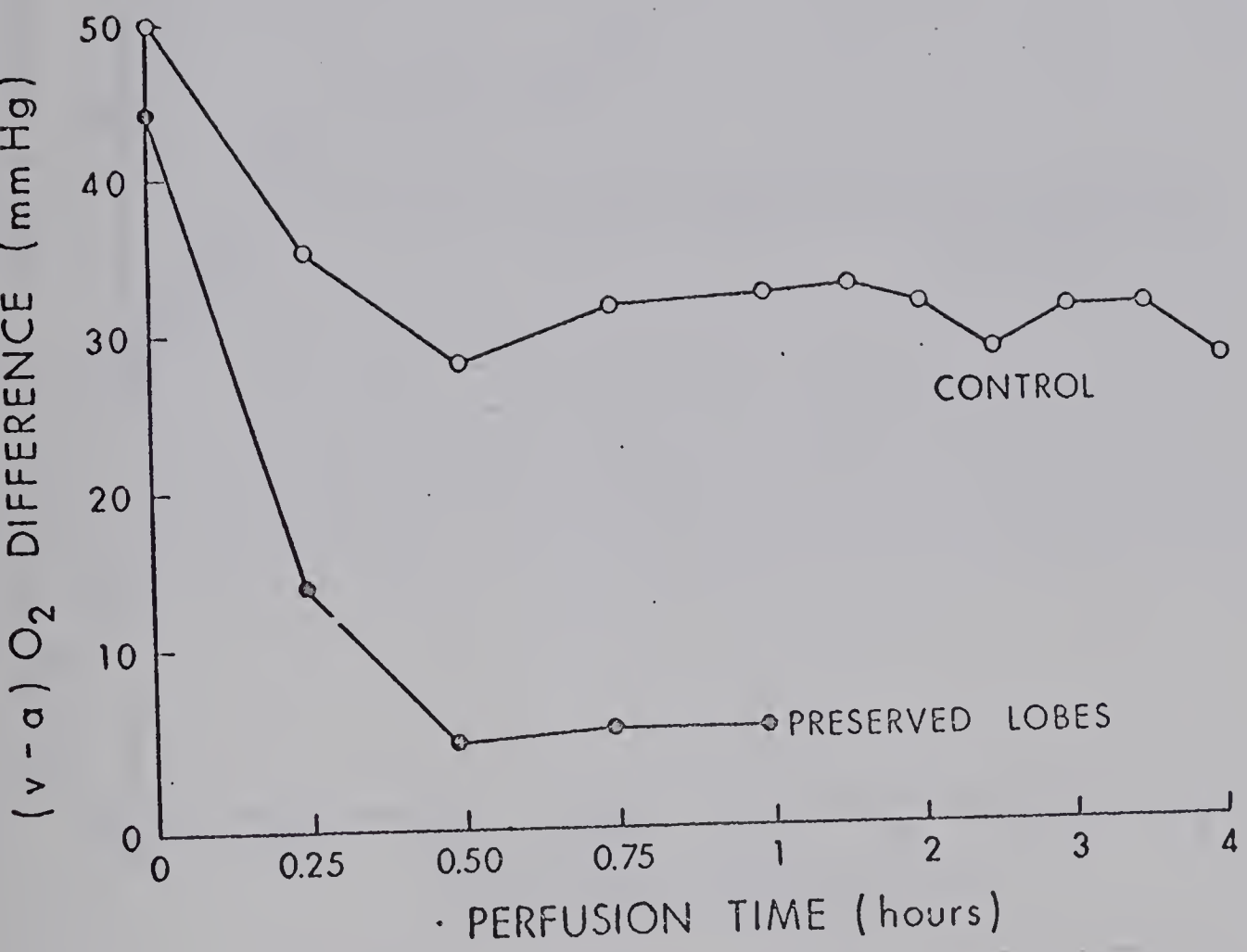


Fig. 4. Venoarterial oxygen differences during perfusion of control and 24 hour stored pulmonary lobes.

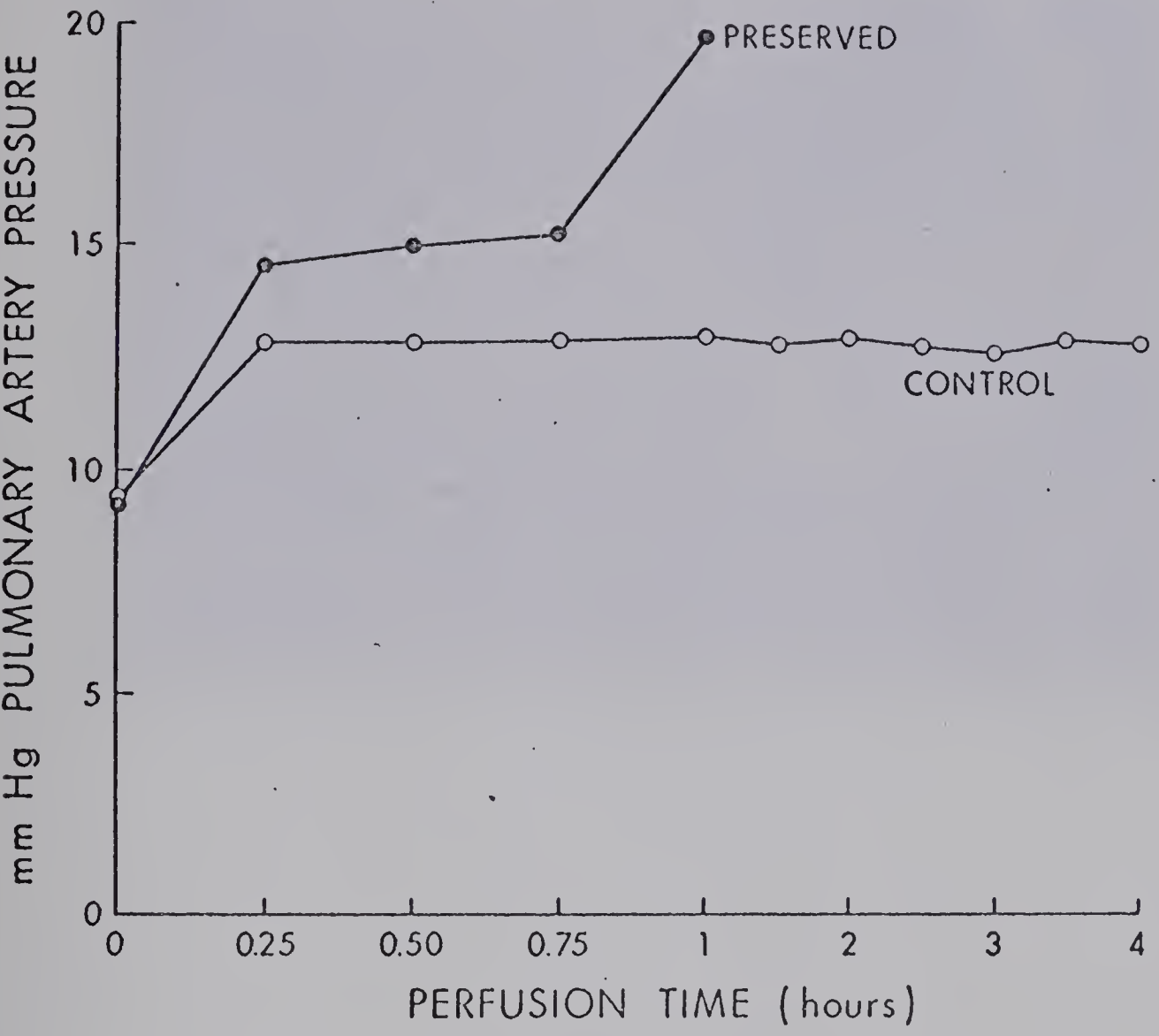


Fig. 5. Pulmonary artery pressure changes during perfusion of control and 24 hour stored lobes.

Fig. 6. Left lower pulmonary lobes stored for 24 hours (hypothermia and hyperbaria) after 30 minutes perfusion.

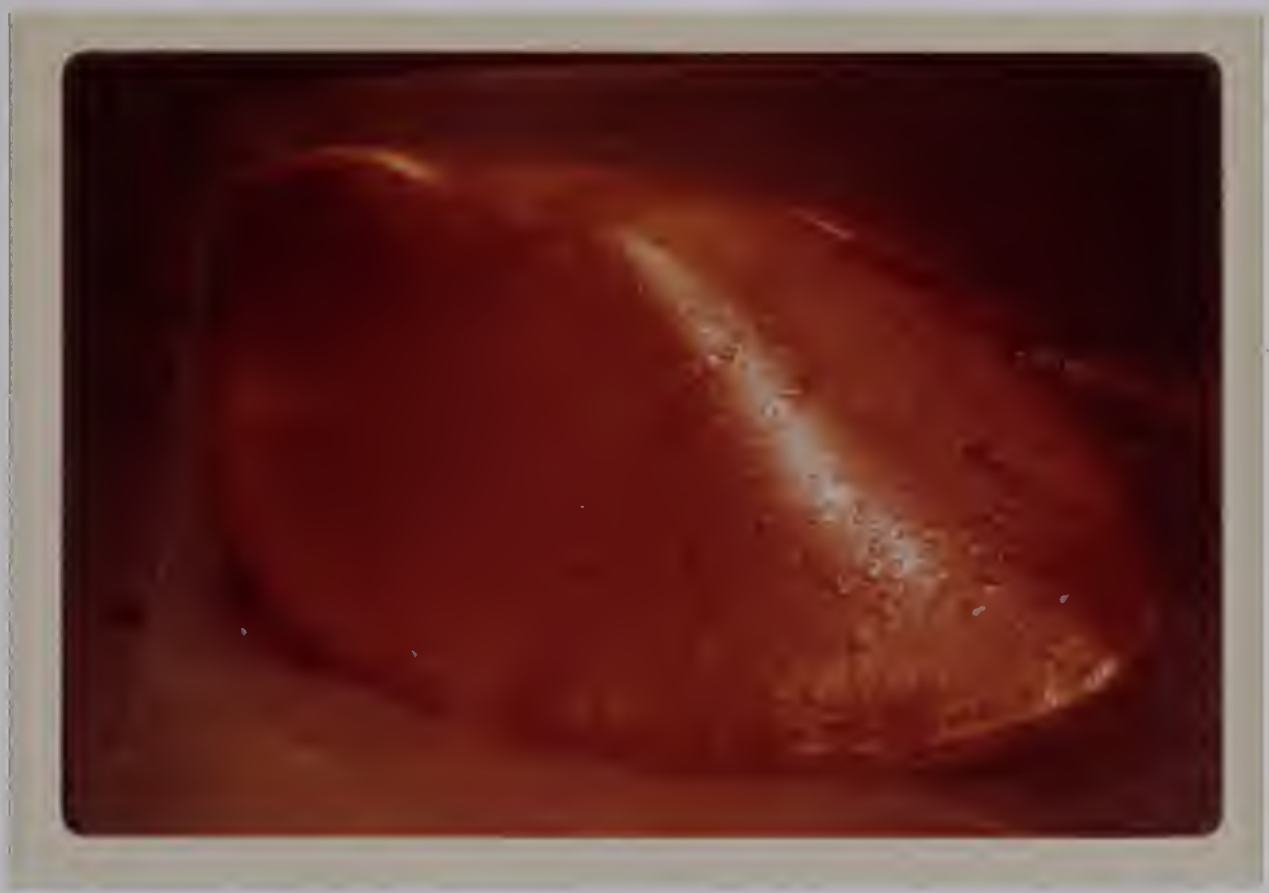


Fig. 7. Control lobe after four hours perfusion.

Fig. 8. Typical photomicrograph (H&E; x175) of pulmonary lobe after 24 hours hypothermic hyperbaric storage. Note well maintained normal pulmonary architecture.

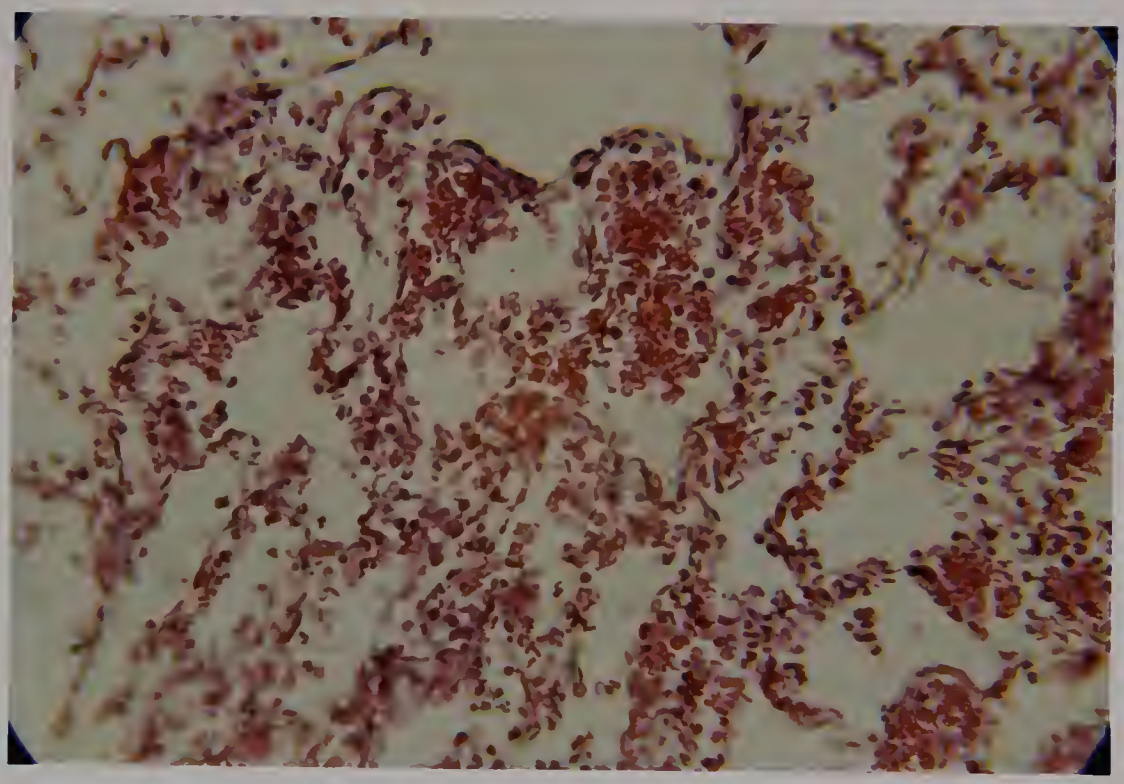


Fig. 9. Typical photomicrograph (H&E; x175) of 24 hour stored pulmonary lobe after 60 minutes perfusion. Note extensive intra-alveolar hemorrhage and microalveolar collapse.

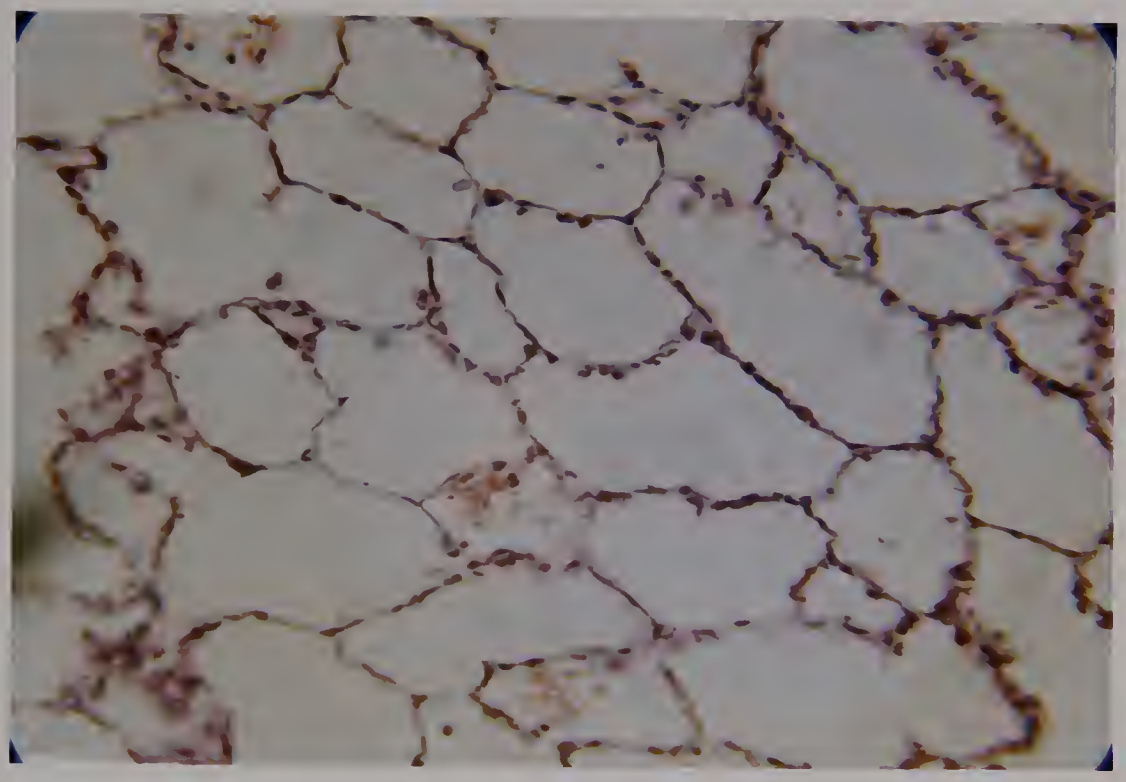


Fig. 10. Typical photomicrograph (H&E; x175) of control pulmonary lobe after four hours perfusion. Note well maintained alveolar architecture with slight interalveolar thickening and extravasation of red cells.

to 90 minutes of perfusion when pale edema began to pour from the bronchial cannulae, and marked dependent zone congestion and ecchymotic surface discoloration with petechiae characterized the lobes. One of the nine stored lobes retained normal function for nearly 4½ hours before the aforementioned events took place.

The typical gross appearance of a 24 hour stored lobe after 45 minutes of perfusion is contrasted with a routine 4 hour perfusion (control) in Figures 6 and 7. Histologic sections of the preserved lobes revealed relatively normal pulmonary architecture immediately after the storage interval (Fig. 8). However, sections of these lobes after only 60 to 90 minutes of perfusion consistently revealed interalveolar thickening and edema, vascular congestion, microvascular collapse and intra-alveolar aggregates of red cells (Fig. 9). Representative sections of control lobes after four and five hours of perfusion revealed changes of much lesser severity (Fig. 10).

Discussion

In this study, stored left lower pulmonary lobes did not function as well as nonpreserved ones, and were undoubtedly inadequate for transplantation. It is noteworthy that the microscopic integrity of the lobes following hypothermic hyperbaric storage did not assure good function. Tissue culture technics have demonstrated that cells which are stored using methods similar to those which we used for the intact organ, and even cells subjected to deep hypothermia[98] are viable and will duplicate. This would seem to indicate, as others have suggested,[99] that the damage inflicted by storage technics may take place at a level of organization functionally higher than the cell. It is possible that

lobes which were stored by the method used here, when transplanted into healthy animals, would function no better in the immediate postoperative period. Late recovery of function under physiologic conditions is possible as shown by other investigators.[95,96]

The ultimate test of a preservation technic is the fully adequate function of the organ immediately following transplantation. The intolerance of pulmonary lobes to hypothermic hyperbaric storage in this study suggests that an objective means of evaluating function after preservation is necessary. The perfusion of preserved lungs is one means of assessing their function and comparing technics.

Prolonged anoxia is the most likely cause of impaired function of stored organs, although lack of other nutrients may be just as vital. Although the empiric success of hyperbaric oxygen in organ preservation has been well documented, high concentrations of oxygen are known to be toxic to lung tissue.[81,82] Furthermore, little attention has been paid to maintenance of the osmotic environment of the lung. Many investigators flush or immerse the lung using hypotonic electrolyte solutions. With attention paid to these details and with objective evaluation of post-preservation lung function, further advances in successful storage of this organ may be expected.

Summary and Conclusions

1. Transplanted lungs must be capable of near normal function immediately following transplantation.
2. The adequacy of lung storage technics is difficult to assess objectively following reimplantation or allografting.

3. The immediate function after a current method of lung storage is inadequate for transplantation.
4. A lung perfusion system is useful in objectively evaluating post-preservation function.

CHAPTER IV

EXTENDED PERFUSION OF THE ISOLATED CANINE LUNG

If current nonperfusion preservation of the isolated lung is unsuccessful in maintaining functional adequacy of the lung for 24 hours, perfusion deserves investigation in this regard. Perfusion studies with isolated lungs have, however, proven more difficult than those with systemic organs.[71,72,100] The longest lung perfusion series in the literature have been routinely successful for only five or six hours, and most workers have described functional deterioration of the lung with gross edema, congestion and atelectasis[101] much earlier. The work of Permutt,[46] Banister,[47] West[48] and others has outlined the delicate pulmonary hemodynamics and normal intrathoracic conditions which must be maintained if long-term perfusion studies of the lung are to be realized.

Fisk and colleagues,[71,72] using the University of Alberta organ perfusion apparatus, have been uniquely successful in perfusing left lower pulmonary lobes routinely for over five hours with well maintained function and morphology. It was felt that further careful simulation of normal conditions with minimal metabolic insult could result in significant extension of this perfusion interval.

Methods

Left lower pulmonary lobes were removed from nine healthy dogs weighing 15 to 32 kgm in the manner described earlier and were perfused in a circuit incorporating the donor animal as deoxygenator (as in Fig. 2). Especial care was taken:

(1) Clean but not sterile technic was followed to minimize bacterial contamination of the circuit.

(2) Handling of the left lung during surgery was minimal.

(3) Complete hemostasis was obtained in the donor animal with the use of electrocautery.

(4) The removed left lower lobe was not flushed with an electrolyte solution.

(5) Anoxia time from clamping of the pulmonary artery to institution of perfusion was less than 12 minutes.

(6) Chamber temperature was accurately maintained at 38°C.

(7) Blood flow to the isolated lobe was begun gradually and increased to a constant 15 cc/donor kgm/min.[97]

(8) Negative pressure ventilation (10-12/min) with humidified air was individualized to provide gentle complete expansion of the lobe with a functional residual capacity. Usual chamber pressure fluctuations were in the range -9/-4 cm HOH.

(9) The lobe was hyperinflated once every fifteen minutes to a maximum -12 cm HOH end inspiratory pressure.

(10) Pharmacologic agents were not added to the circuit.

(11) The donor animal was maintained with accurate fluid replacement and careful attention to arterial blood gases.

Measurements

Pulmonary arterial, venous and donor arterial gases and pH were determined every 30 minutes throughout the perfusion; the corresponding pressures were monitored continuously. Lobe tidal volumes and ventilating pressures were also recorded every 30 minutes. The weight of each lobe was continuously recorded during each perfusion. Representative sections of the perfused lobes and lobes from the support animal were fixed in

formalin at the end of the perfusion for histologic examination.

Results*

Table III presents data in eight lobes which were perfused for a minimum of 12 hours. One perfusion was discontinued after 5½ hours of excellent function due to uncontrolled hemorrhage in the support animal and is not included in this group. The remaining eight lobes were perfused from 12 to 18 hours with a mean perfusion time of 14.4 hours. After 12 hours of perfusion, hypoxia of the support animal and falling arterial pressure with continued oozing from the thoracotomy wound ultimately forced termination of all perfusions. During the initial 12 hours of perfusion however, isolated lobe function was excellent and in this interval no drugs were added to the system. Pulmonary arterial pressure (Fig. 11) and pulmonary vascular resistance (Fig. 12) rose slowly over the course of the experiments and compliance diminished gradually (Fig. 13). Gas transfer was grossly adequate during this interval as reflected by a mean venoarterial oxygen gradient of 55 mm Hg after 12 hours of perfusion (Fig. 14). Studies with 100% oxygen were not done, since high alveolar oxygen tensions can produce pulmonary alteration.[102]

Gross edema did not occur in any of the lobes. Weight of each lobe was continuously monitored throughout the perfusion interval, and the mean weight gain after 12 hours of perfusion for the eight lobes was only 31%. Distinction between blood and extravascular fluid volume could not be made.

A photograph of a left lower lobe after 10 hours of perfusion is presented in Fig. 15, and illustrates characteristic normal color and

---

*Sample individual tidal volumes and ventilating pressures are recorded in Appendix III.

TABLE III - AVERAGE PULMONARY HEMODYNAMICS AND FUNCTION OF EIGHT LEFT PULMONARY LOBES PERFUSED FOR 12 HOURS

Time of Perfusion	Pulmonary Arterial Pressure (mm. Hg)	Pulmonary Vascular Resistance* (dynes sec. cm.$^{-5}$)	Lobe Compliance (cc./cm. HOH/ lobe g.)x 100	(A-V) CO_2 Difference (mm. Hg)	(V-A) O_2 Difference (mm. Hg)
0	7.8	5250	29.8	32.3	64.5
1	9.8	3120	31.8	9.3	67.0
2	11.4	3860	28.2	10.6	56.9
3	11.1	3820	33.9	9.5	62.5
4	11.8	3940	31.2	11.9	60.3
5	13.8	5200	28.5	9.4	52.4
6	13.9	5070	25.5	10.6	54.1
7	14.9	5330	26.4	11.8	58.1
8	15.3	5310	24.4	10.4	60.3
9	15.0	5230	26.0	9.8	61.6
10	14.6	6370	23.9	12.0	61.1
11	16.3	6140	25.7	8.9	57.9
12	17.8	6630	21.6	8.5	54.9

* $PVR = \frac{(PA-PV) \times 1332}{Flow} \times 60$

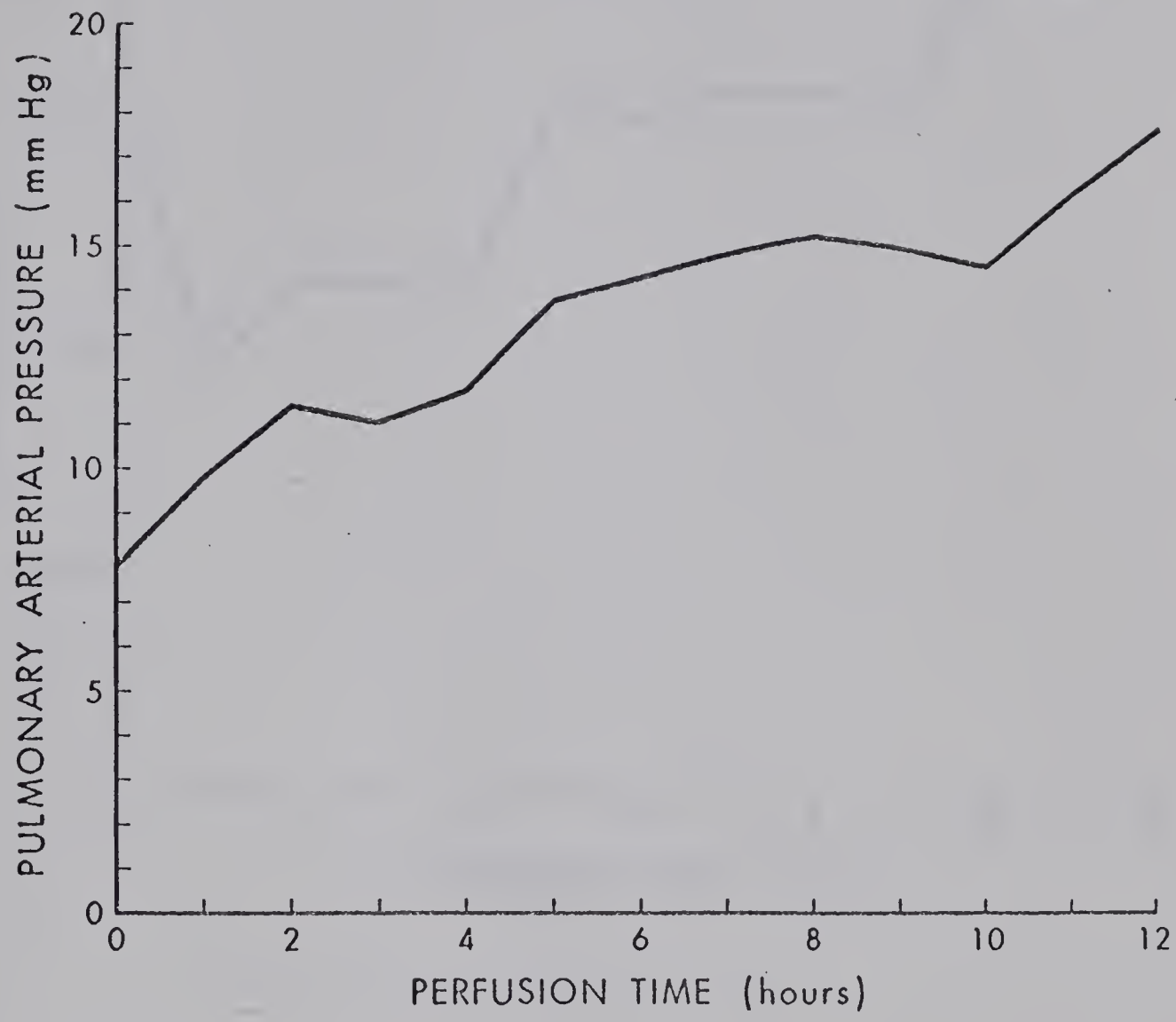


Fig. 11. Mean pulmonary artery pressure changes over 12 hour perfusion (eight lobes).

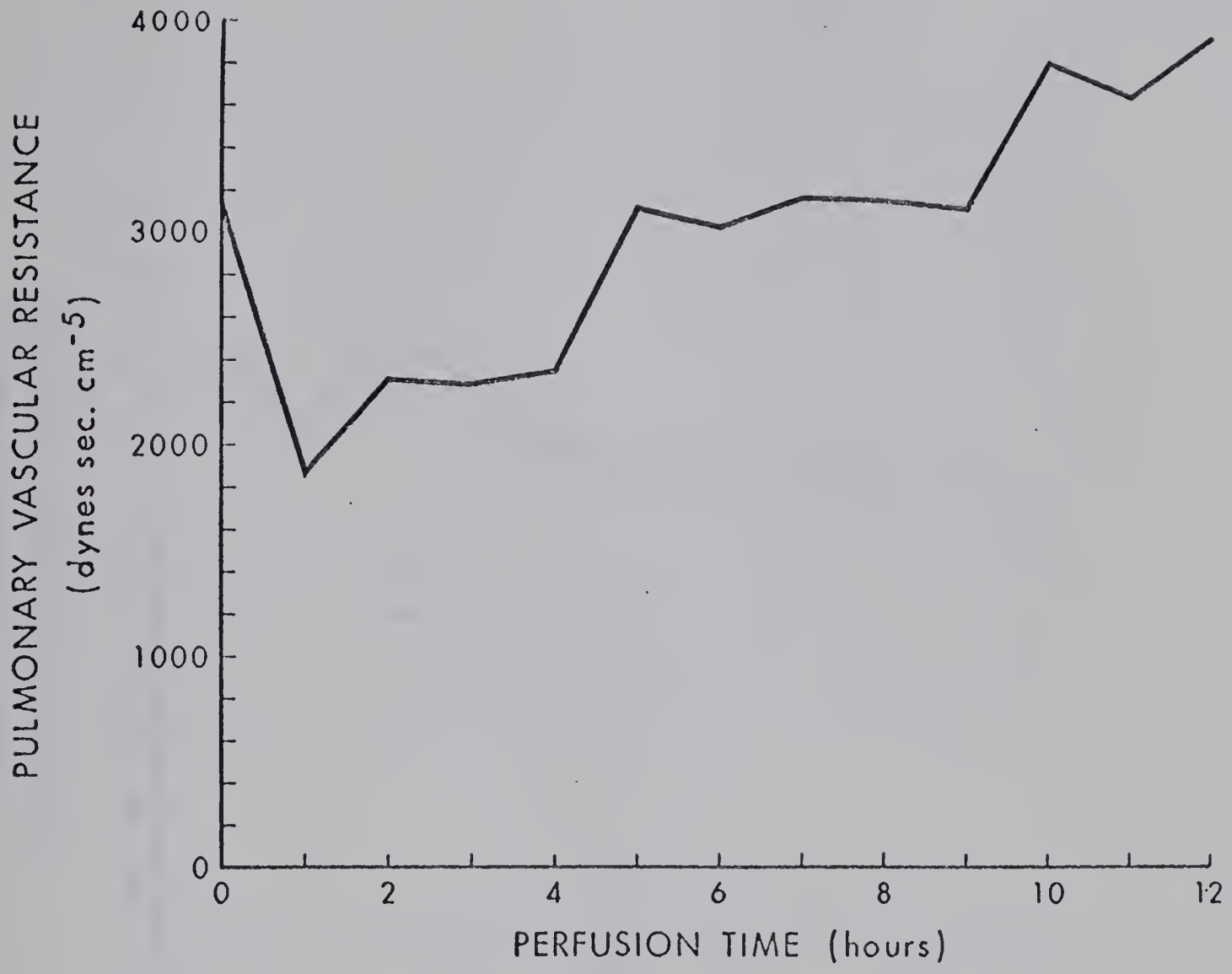


Fig. 12. Pulmonary vascular resistance changes in eight lobes perfused for 12 hours.

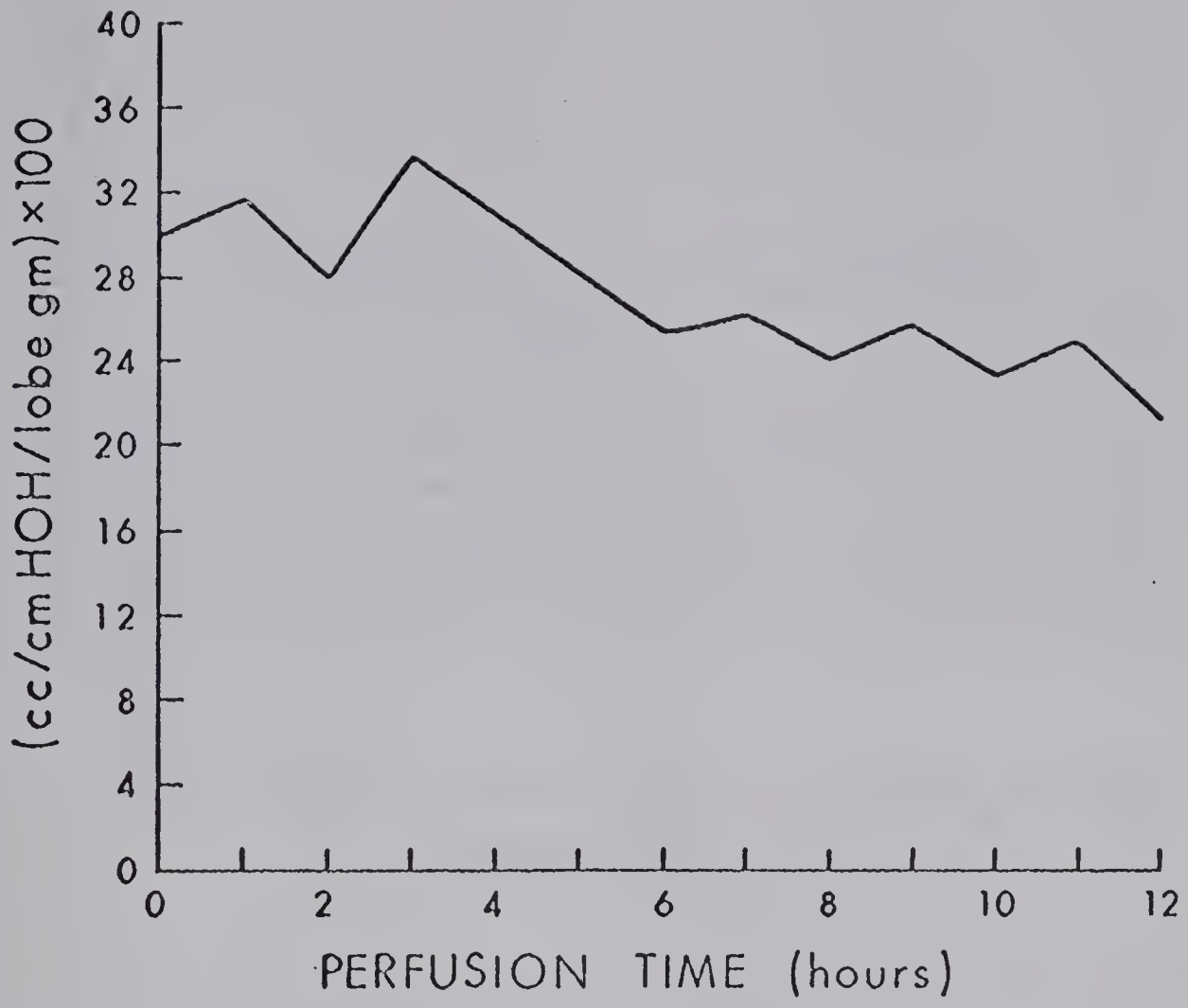


Fig. 13. Mean compliance in 12 hour perfusion of eight pulmonary lobes.

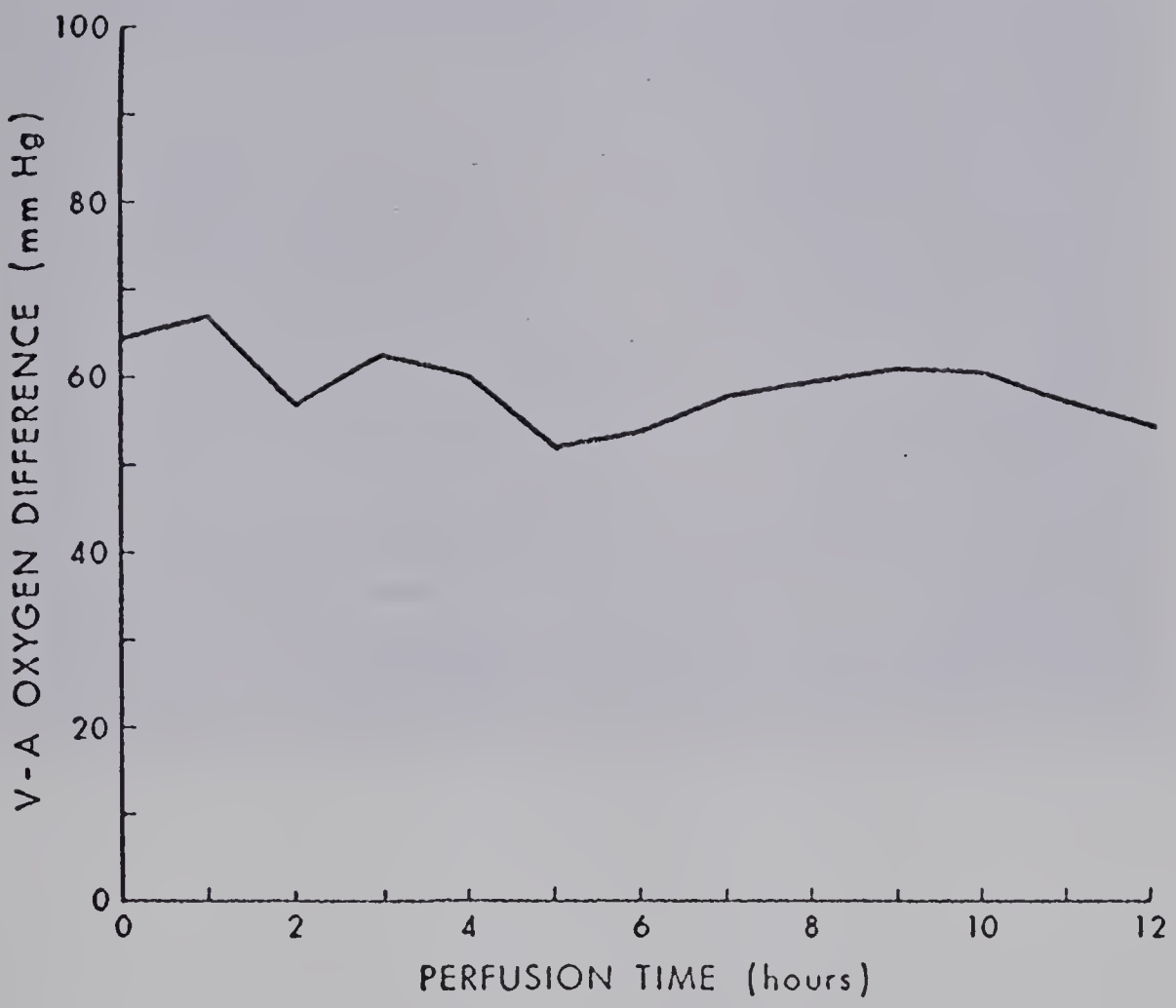


Fig. 14. Mean venoarterial oxygen differences during 12 hour perfusion of eight pulmonary lobes.

Fig. 15. Isolated left lower lobe after ten hours ex vivo perfusion.

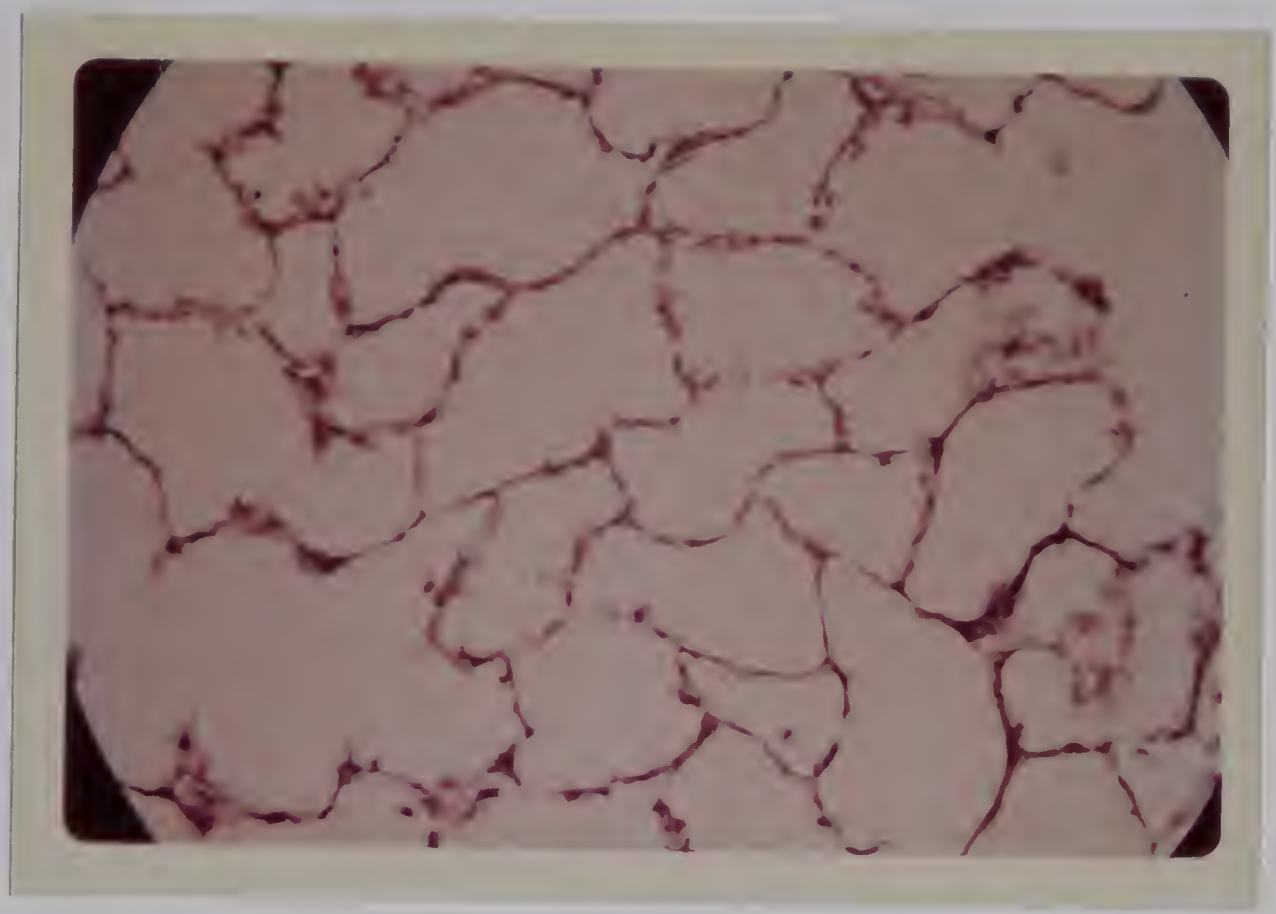


Fig. 16. Typical photomicrograph (H&E; x175) of left lower lobe perfused for 14 hours. Note well maintained alveolar architecture, mild interalveolar edema and exudate.

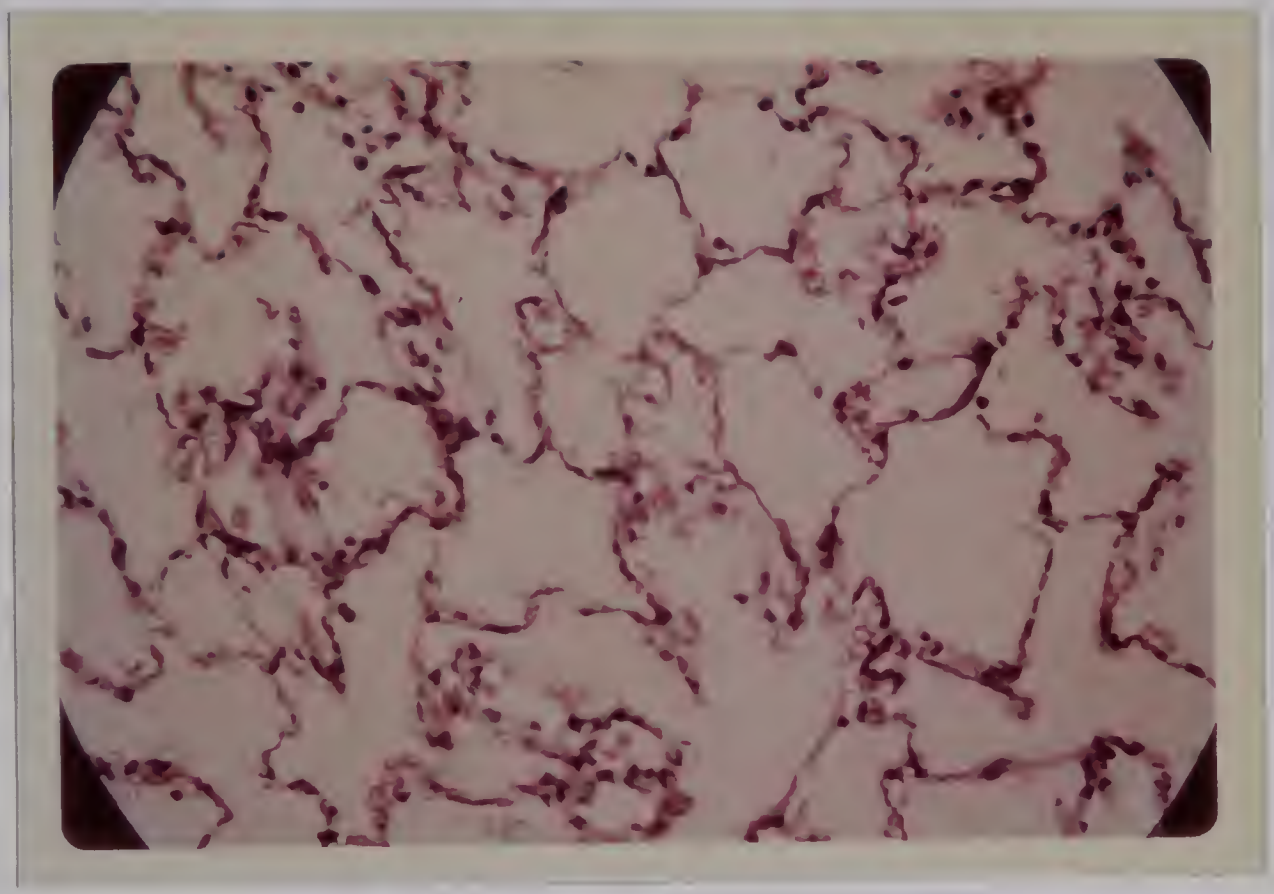


Fig. 17. Typical photomicrograph of donor lung subjected to positive pressure ventilation for 12 hours. (H&E; x175)

Note: microalveolar collapse, congestion and intra-alveolar red cells.

absence of subpleural hemorrhage. Microscopic evaluation of lobes perfused for 12 hours or more indicated mild interstitital edema, leucocytic infiltration and capillary congestion but well-maintained alveolar architecture (Fig. 16). In contrast to this, lobes removed from support animals which had been exposed to intermittent positive pressure ventilation for the same period of time, were bluish-red in color and exhibited marked dependent edema. Histologic sections from these lobes (Fig. 17) revealed more marked interalveolar edema and exudate, vascular congestion and microvascular collapse with perivascular hemorrhage.

Discussion

Increased success in maintaining the function of perfused lobes in this experiment was due to closer simulation of the normal pulmonary environment with avoidance of dehydration, direct trauma and prolonged anoxia. Although negative pressure ventilation has not been proven less harmful than positive pressure in isolated perfusion experiments, ventilation with positive pressure is unphysiologic and often results in over ventilation.[103] As well, lack of a functional residual capacity contributes to alveolar collapse and progressive atelectasis, conditions in which capillary resistance is elevated.[45] Maintaining physiologic flow is undoubtedly important at normothermia[104] and the role of the venous pressure in maintaining the microcirculation has recently been emphasized by Fisk,[72] Dritsas[73] and Couves.[74] Flow in this perfusion system, though semi-pulsatile, does not have the pulse characteristics of the normal pulmonary artery and invites further work.

The importance of support (donor) animals in these experiments is

evident, for each serves as a convenient deoxygenator for arterialized blood from the pulmonary artery and also as a filter for microemboli known to be important in blood perfusion systems. The use of a support animal also provides a more constant nutrient environment, promotes acid-base balance, and probably acts as a bacterial filter and a metabolizer for vasoactive substances[28] released from the perfused organ. It was observed that the eventual decline in function of the perfused lobe corresponded closely with the terminal development of hypotension and metabolic acidosis in each animal.

Summary and Conclusions

1. Careful simulation of normal physiologic conditions provides an improved lung perfusion system.
2. A method is presented which will routinely preserve function and morphology of the ex vivo lung for periods of 12 hours or longer.
3. The importance of the support animal in these perfusion studies requires further investigation.

CHAPTER V

BIOCHEMICAL ASPECTS OF LUNG PERFUSION

Since perfusion is being explored as a means of maintaining ex vivo organ function, it is evident that, for clinical application, a means of excluding a support animal from the circuit must be found. The support animal probably functions in maintaining perfusate composition and metabolizes toxic substances released from the perfused lung. In order that the biochemical changes occurring in the perfusion circuit without a support animal could be detailed, it was decided to perfuse left lower pulmonary lobes in a closed circuit in which perfusate composition could be tested at precise intervals.

Methods

Eight healthy mongrel dogs weighing from 14 to 29 kilograms were used in this study. Left lower pulmonary lobes were isolated and removed in the usual fashion with simultaneous collection of 1000-1200 cc donor arterial blood in a sterile heparinized collection bottle. The left lower lobe was then cannulated in the perfusion chamber at the same time as the perfusion circuit was primed with heparinized autologous blood. Instead of returning arterialized blood from the perfused lobe to the donor animal, in this series the blood was returned with an occlusive roller pump to a small oxygenator bag* through which varying concentrations of carbon dioxide and nitrogen were bubbled in order to achieve normal venous gas tenions and pH values. Deoxygenated blood was then returned with a second occlusive roller pump to the cannulated

---

* Travenol 2LF Infant Oxygenator.

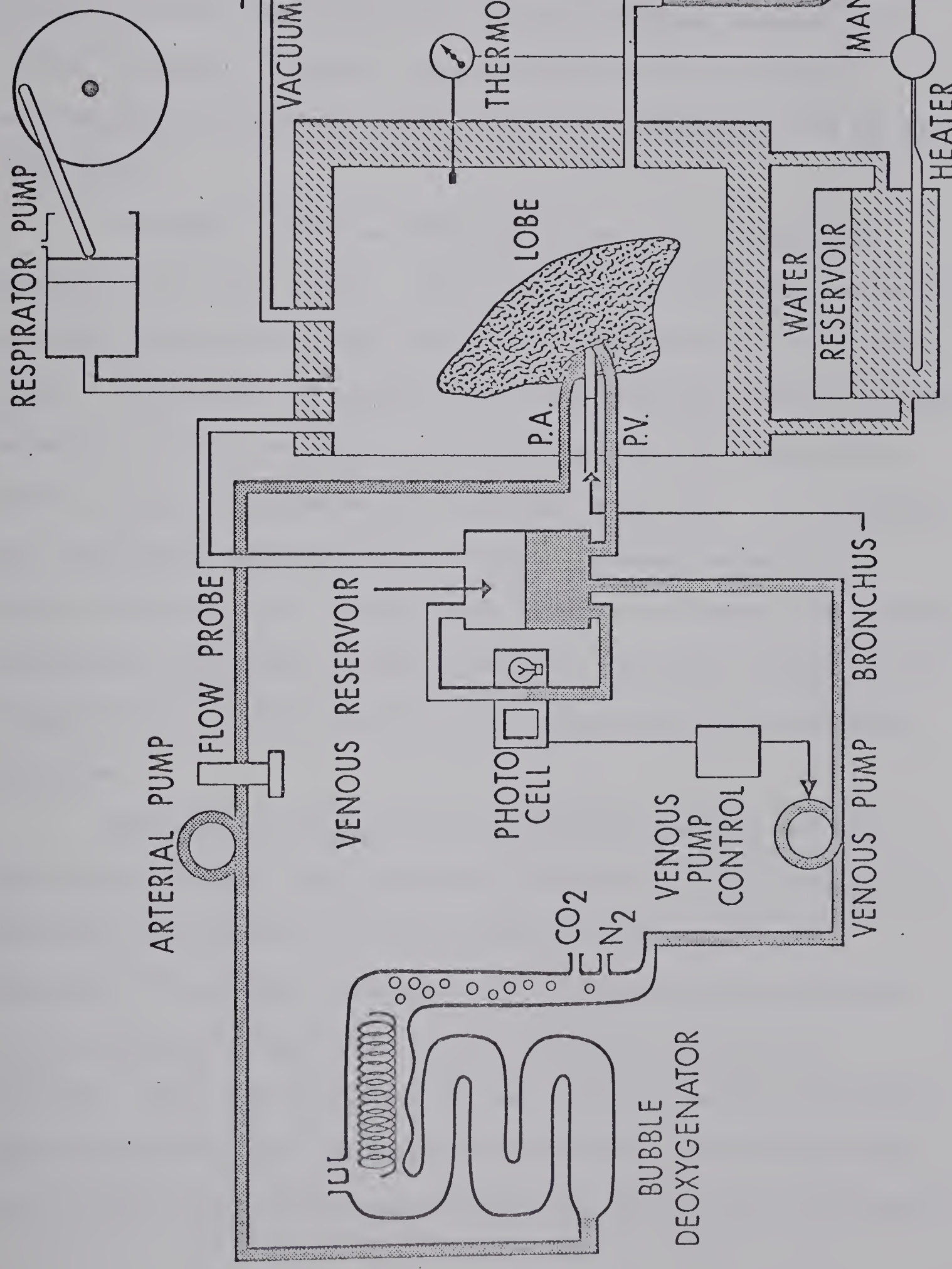


Fig. 18. Schematic representation of lung perfusion circuit incorporating a bubble deoxygenator and excluding the donor animal.

pulmonary artery and the cycle was repeated (Fig. 18). In this manner, a constant volume of recirculating blood was available for repeated sampling. There was no source of blood leakage in the system. Initial hematocrit values ranged from 25 to 38% but individual changes were minimal throughout perfusion. Blood in the circuit was initially anticoagulated with 150 mgm sodium heparin and increments of 50 mgm were added hourly.

Perfusion control was identical to that used in experiments incorporating a donor animal. Flow through the perfused lobe was begun gradually and increased over 15 minutes to a constant 15 cc/donor kgm/minute. Ventilation was accomplished with intermittent negative pressure excursions of -9/-4 cm HOH at a rate of 12/minute and a mean positive venous pressure of approximately 2 mm Hg was maintained. All perfusions were arbitrarily terminated after 5 hours. Pulmonary arterial and venous blood gases were recorded every 30 minutes throughout the perfusion. Simultaneous lobe tidal volumes and negative ventilation pressures were recorded and the weight of each lobe was continuously monitored during perfusion.

Specimens of left upper lobe (nonperfused control) and left lower lobe after five hours perfusion were frozen in liquid nitrogen for subsequent determination of tissue lactate,[105] pyruvate[106] and histamine.[107] Ten cubic centimeters of blood perfusate was collected at the beginning of each perfusion and hourly for five hours of perfusion. This was assayed for glucose, pyruvate, lactate, electrolyte and serotonin[108] content throughout the perfusion. In two experiments 10 cc samples of blood were taken after 0, 2½, and 5 hours of perfusion,

boiled for 10 minutes and centrifuged for 30 minutes at 20,000G. The supernatant was then tested for its contractile stimulatory effect on guinea pig ileum in an electrolyte bath.*

Results¶

Table IV presents the mean hourly changes in perfusate composition for eight lobes (represented graphically in Figs. 19-25). Very rapid depletion of glucose is evident with virtually none remaining after five hours perfusion. In contrast, perfusate lactate and pyruvate rose steadily. Blood potassium remained relatively stable throughout the experiments but serotonin content rose approximately 60%. Tissue pyruvate and lactate (Table VI), also rose remarkably in pre- and postperfusion tissue specimens with three and nine fold increases in each respectively. (All values are corrected for weight changes over the course of each perfusion so that changes can be compared with validity.) In addition, significant amounts of histamine are liberated from the perfused lung since postperfusion tissue histamine content is approximately 35% less than control values. In two experiments in which the perfusate was tested for contractile effect on isolated guinea pig ileum (Appendix IV, page 95), significant smooth muscle stimulation was noted in perfusate drawn after 2½ and 5 hours of perfusion, but not in the initial samples.

The hemodynamic and functional changes in eight lobes are represented in Table VII. It is evident that lobe function as reflected

---

* Appendix IV

¶ Individual sample data for glucose is presented in Appendix V.

TABLE IV - PERFUSATE BIOCHEMISTRY IN EIGHT LOBES PERFUSED WITH AUTOLOGOUS BLOOD FOR FIVE HOURS

(RECIRCULATION WITH BUBBLE DEOXYGENATION)

Time (hours)	Glucose (mgm./100 ml.)	Pyruvate (mgm./100 ml.)	Lactate (mgm./100 ml.)	Potassium (meg./litre)	Serotonin (µgm./ml.)
0	76 ± 25	0.63 ± 0.17	14.8 ± 6.7	3.5 ± 0.3	0.12 ± 0.04
1	57 ± 23	1.27 ± 0.24	28.9 ± 7.8	3.6 ± 0.2	0.11 ± 0.02
2	35 ± 10	1.55 ± 0.46	34.6 ± 8.7	3.6 ± 0.2	0.13 ± 0.03
3	22 ± 11	1.98 ± 0.57	45.3 ± 9.1	3.6 ± 0.3	0.14 ± 0.03
4	14 ± 9	2.38 ± 0.49	55.9 ± 10.0	3.5 ± 0.2	0.16 ± 0.09
5	17 ± 4	2.56 ± 0.49	65.2 ± 13.0	3.6 ± 0.4	0.19 ± 0.13

TABLE V - PULMONARY TISSUE HISTAMINE CHANGES DURING FIVE HOURS PERFUSION

Expt.	Initial Weight (grams)	Weight Post-Perf. (grams)	Weight Gain %	Tissue Histamine Pre-Perf. µgm./g. Tissue	Tissue Histamine Post-Perf. µgm./g. tissue	Corrected Tissue Histamine Post-Perf. µgm./g. tissue
L-2	89	189	112	23.4	12.5	26.2
L-3	132	162	23	34.4	21.0	25.8
L-5	101	168	67	27.4	10.5	17.5
L-6	84	104	24	25.8	13.7	16.9
L-7	99	112	13	38.3	16.5	18.6
L-8	94	149	59	20.2	15.6	24.8
L-9	88	128	43	23.5	13.7	19.6
L-10	127	152	18	21.9	21.8	25.7
Mean	101.7	145.5	44.8	27.4 ±6.6	15.6 ±4.0	20.4 ±3.7

TABLE VI - PULMONARY TISSUE LACTATE AND PYRUVATE CHANGES DURING FIVE HOURS PERFUSION

Expt.	Initial Weight (grams)	Weight Post-Perf. (grams)	Weight Gain %	Tissue Lactate Pre-Perf. µmoles/g. Tissue	Tissue Lactate Post-Perf. µmoles/g. Tissue	Corrected Lactate Post-Perf. µmoles/g. Tissue	Tissue Pyruvate Pre-Perf. µmoles/g. Tissue	Tissue Pyruvate Post-Perf. µmoles/g. Tissue	Corrected Pyruvate Post-Perf. µmoles/g. Tissue
L-2	89	189	112	ND	ND	ND	ND	ND	ND
L-3	132	162	23	ND	ND	ND	ND	ND	ND
L-5	101	168	67	0.67	8.34	13.94	0.09	0.16	0.27
L-6	84.7	104	24	1.04	7.05	8.74	0.43	0.68	0.84
L-7	99	112	13	1.29	3.67	4.15	0.19	0.49	0.55
L-8	94	149	59	0.75	4.49	7.14	0.04	0.22	0.35
L-9	88	128	43	0.62	5.39	7.71	0.04	0.22	0.31
L-10	127	152	18	1.02	5.85	6.90	0.18	0.36	0.42
Mean	101.7	145.5	44.8	0.90 ±0.26	5.80 ±1.70	8.10 ±3.24	0.16 ±0.14	0.36 ±0.20	0.46 ±0.21

ND = Not Done

TABLE VII - AVERAGE FUNCTION AND HEMODYNAMICS OF EIGHT PULMONARY LOBES PERFUSED WITH AUTOLOGOUS BLOOD FOR FIVE HOURS (RECIRCULATION WITH BUBBLE DEOXYGENATION)

Time (hours)	Pulmonary Arterial Pressure (mm Hg)	Pulmonary Vascular* Resistance (dynes sec. $cm.^{-5}$)	Lobe Compliance (cc/cm HOH/lobe g. X 100)	(A-V) CO_2 Difference (mm Hg)	(V-A) O_2 Difference (mm Hg)
0	10.5	4990	38.3	28.3	43.3
1	13.5	3130	47.2	15.5	56.4
2	13.4	3120	40.4	14.9	57.0
3	12.3	2880	40.5	12.0	50.1
4	12.0	2650	36.7	10.4	49.5
5	12.1	2680	34.0	16.5	50.5

*$PVR = \frac{(PA-PV) \times 1332}{Flow} \times 60$

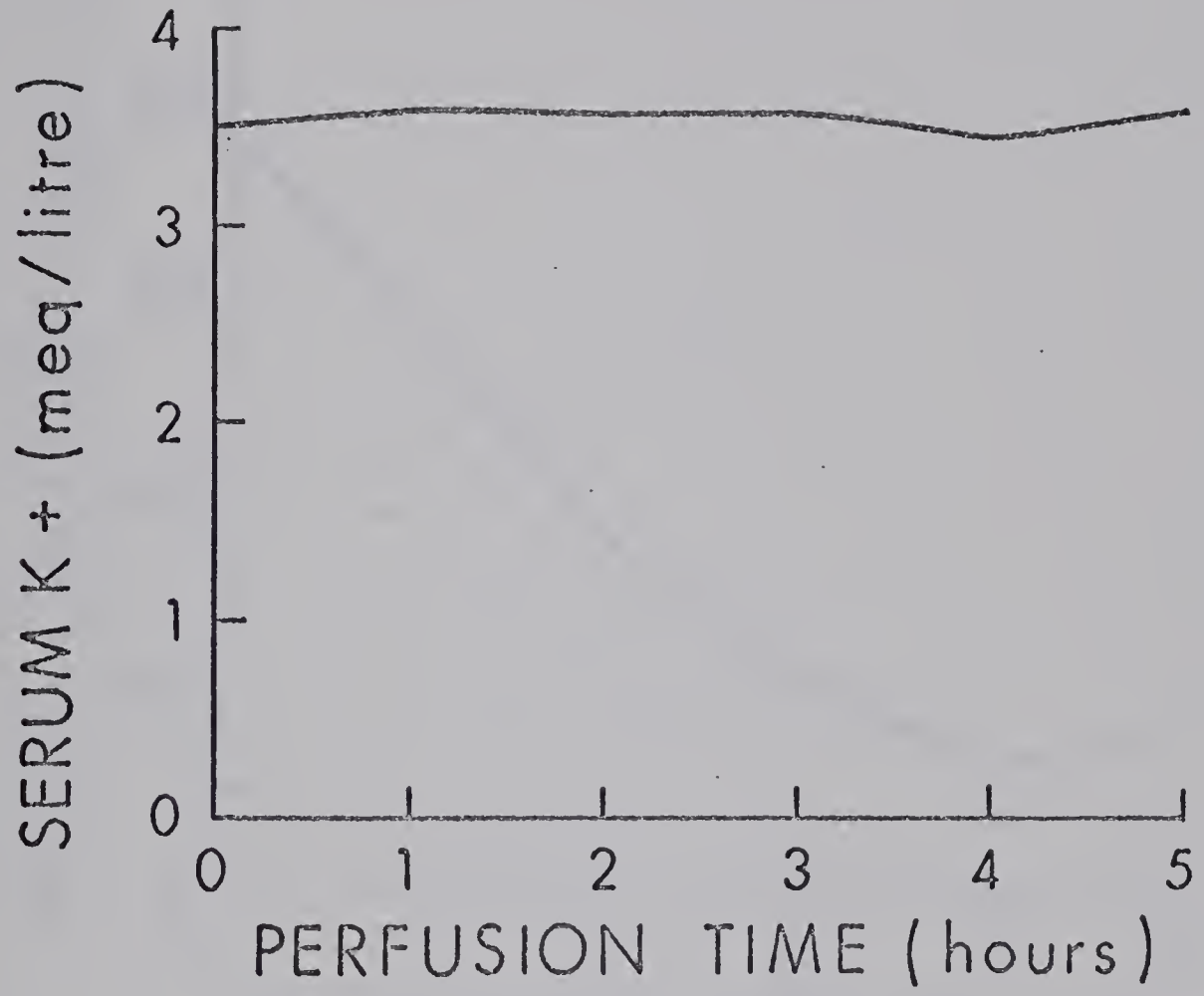


Fig. 19. Perfusate potassium levels during five hours perfusion.

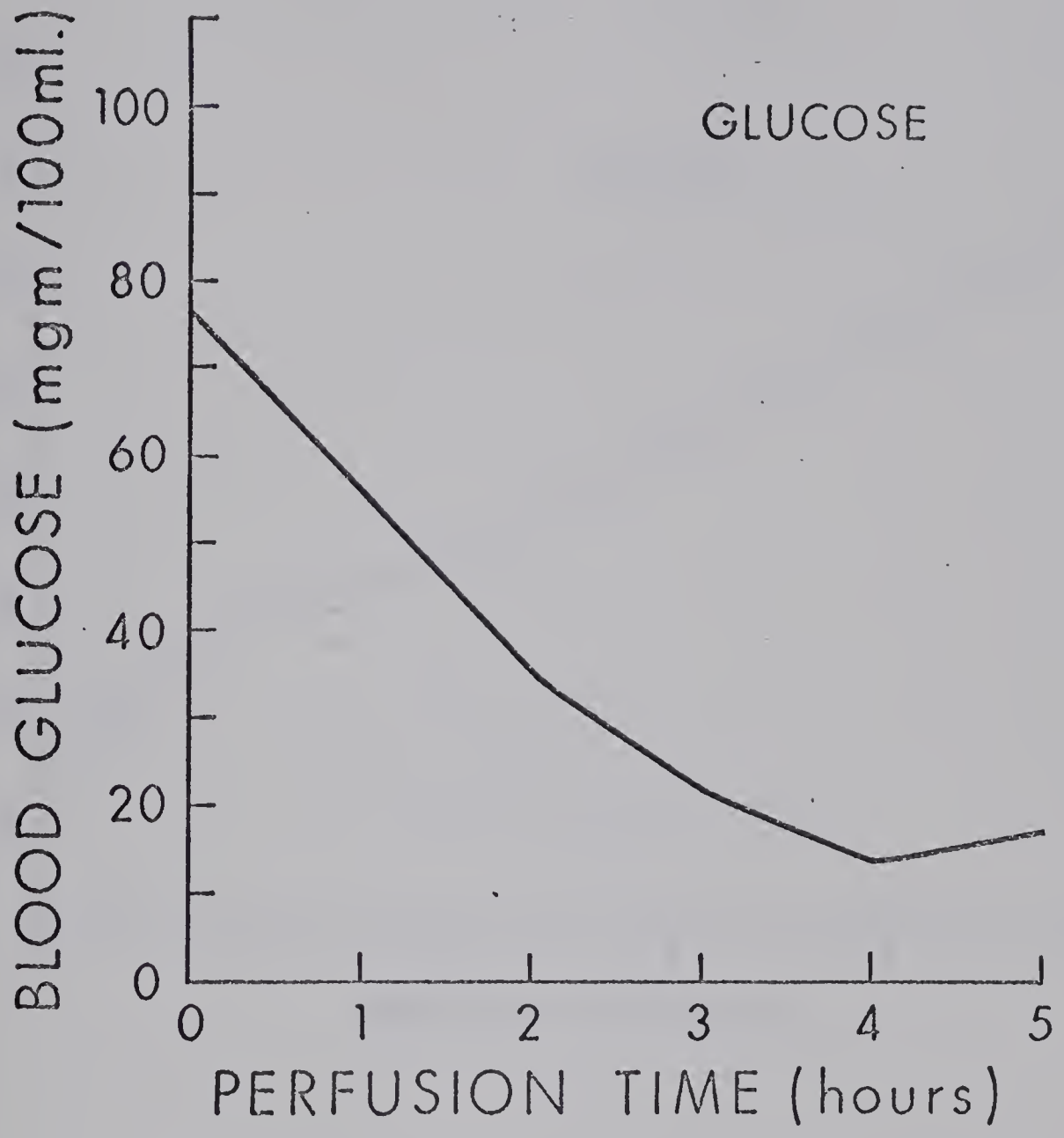


Fig. 20. Perfusate glucose levels during five hours perfusion.

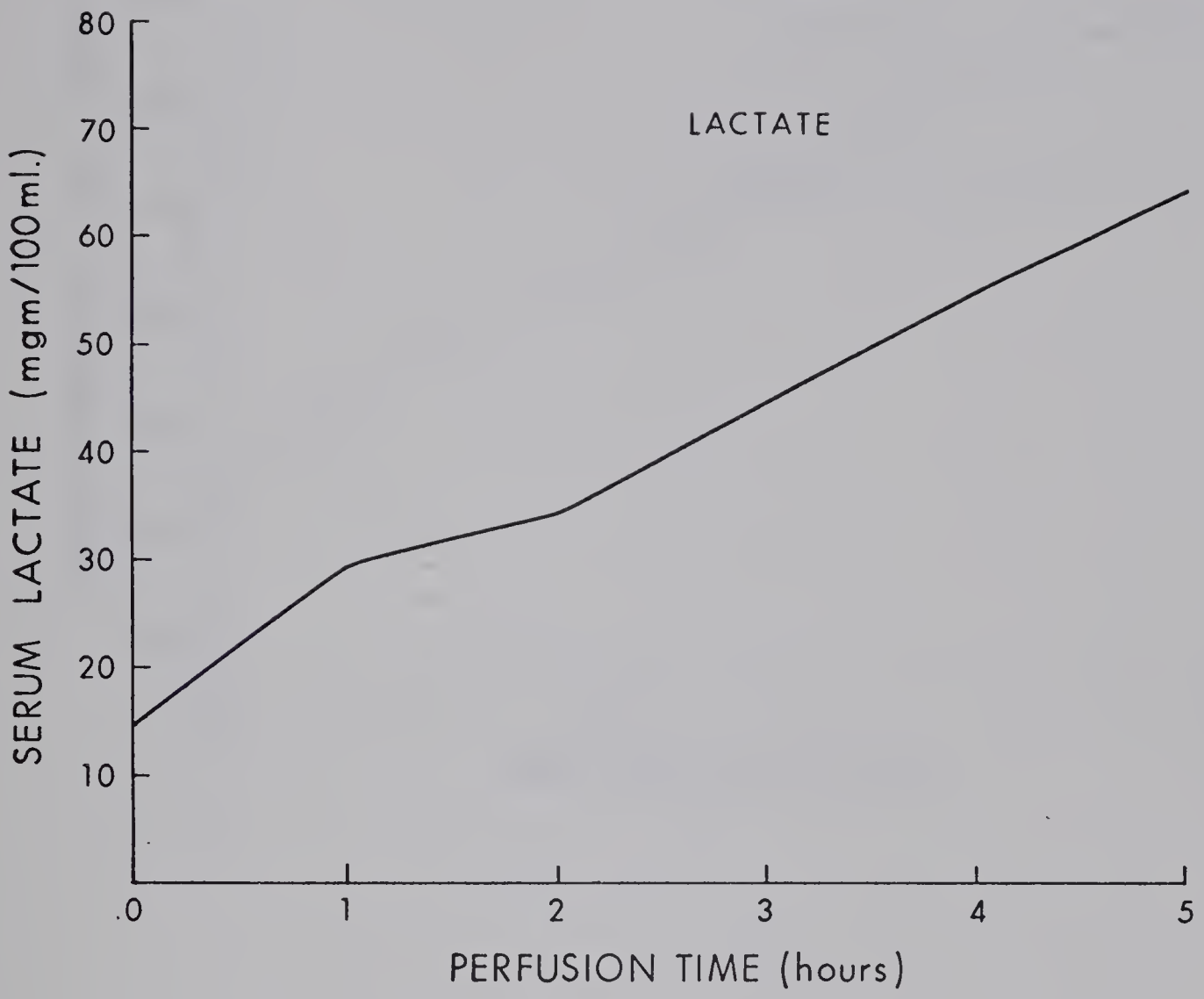


Fig. 21. Perfusate lactate levels during five hours perfusion.

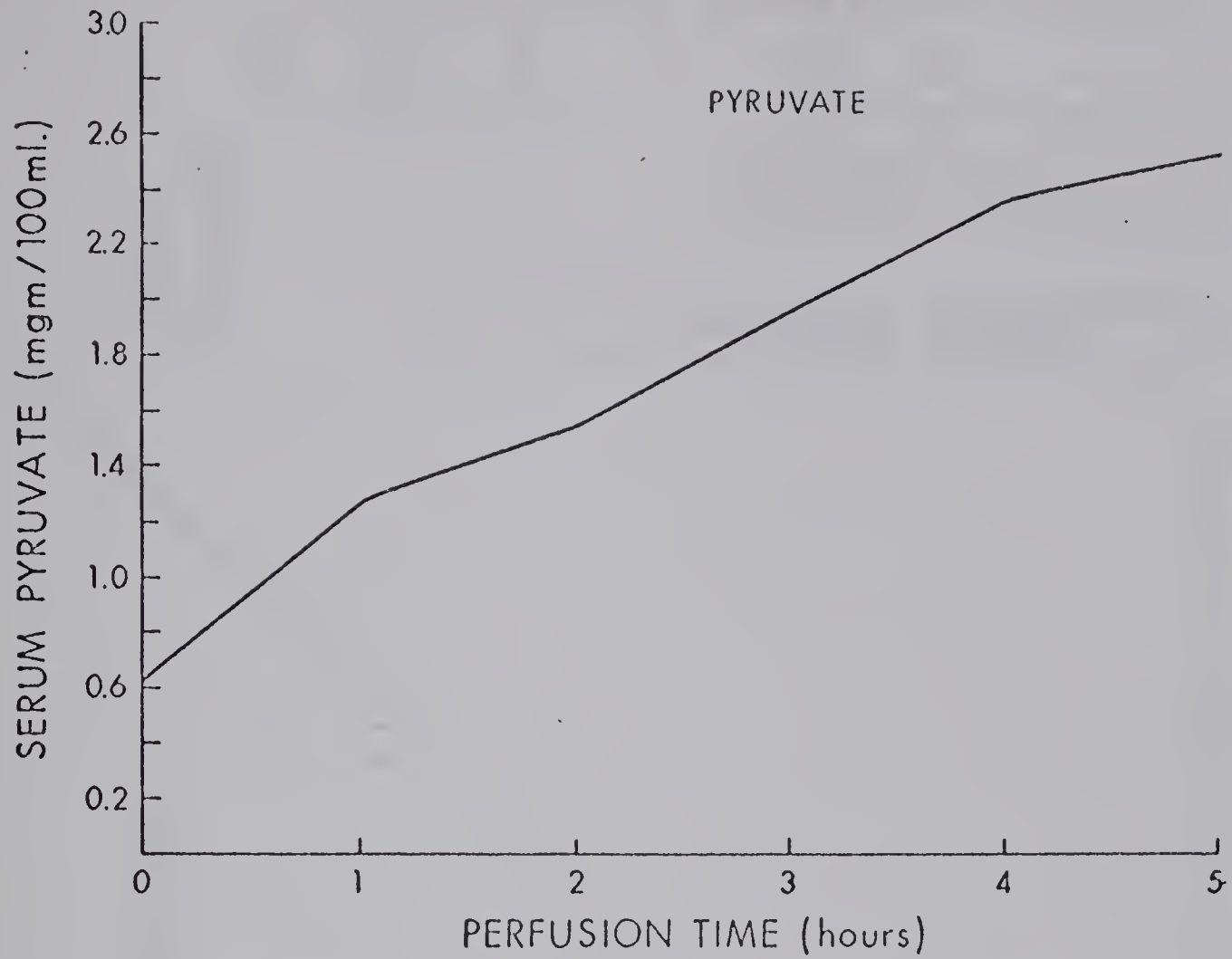
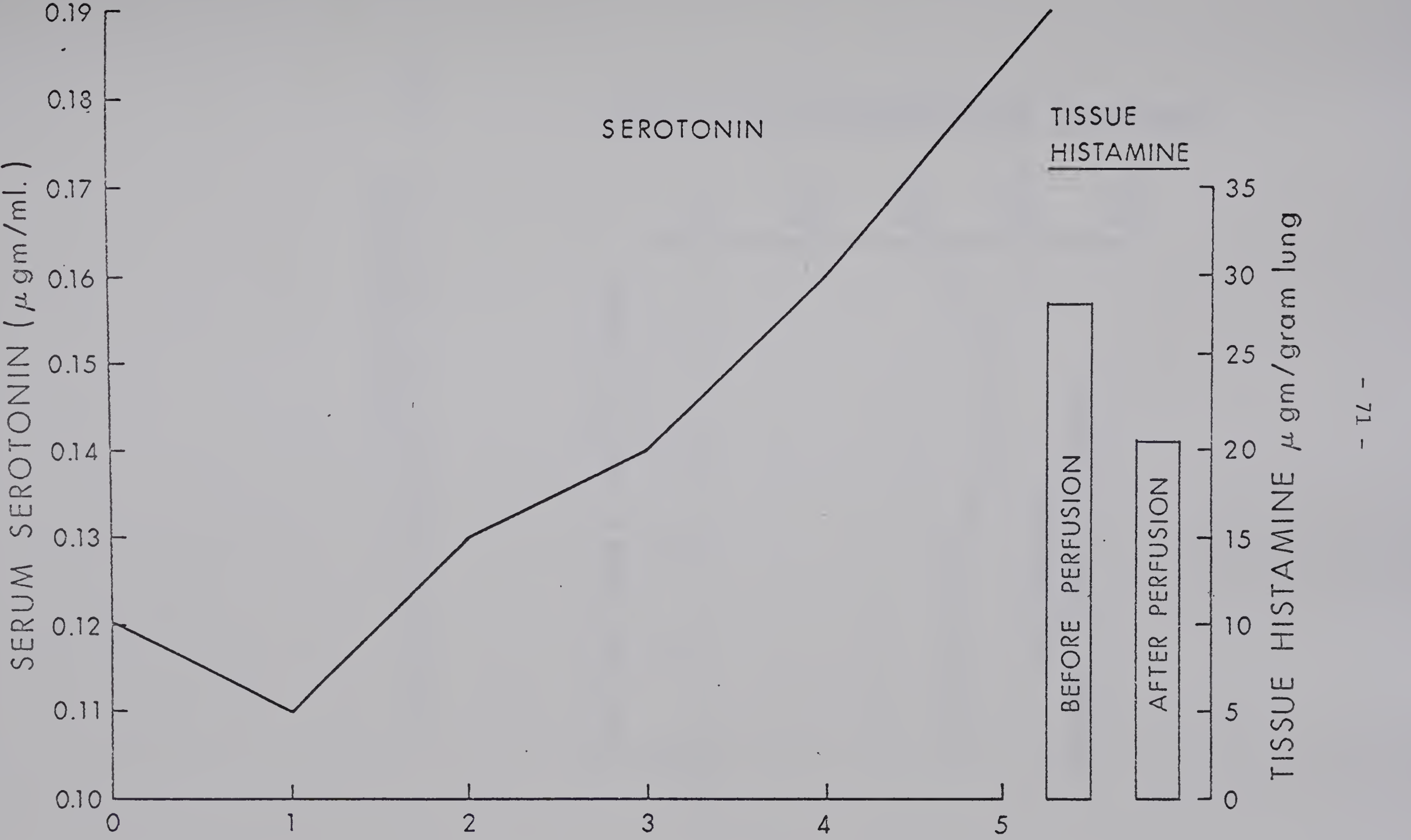


Fig. 22. Perfusate pyruvate levels during five hours perfusion.


SEROTONIN
TISSUE HISTAMINE
SERUM SEROTONIN (μgm/ml.)
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0
1
2
3
4
5
TISSUE HISTAMINE μgm/gram lung
35
30
25
20
15
10
5
0
BEFORE PERFUSION
AFTER PERFUSION

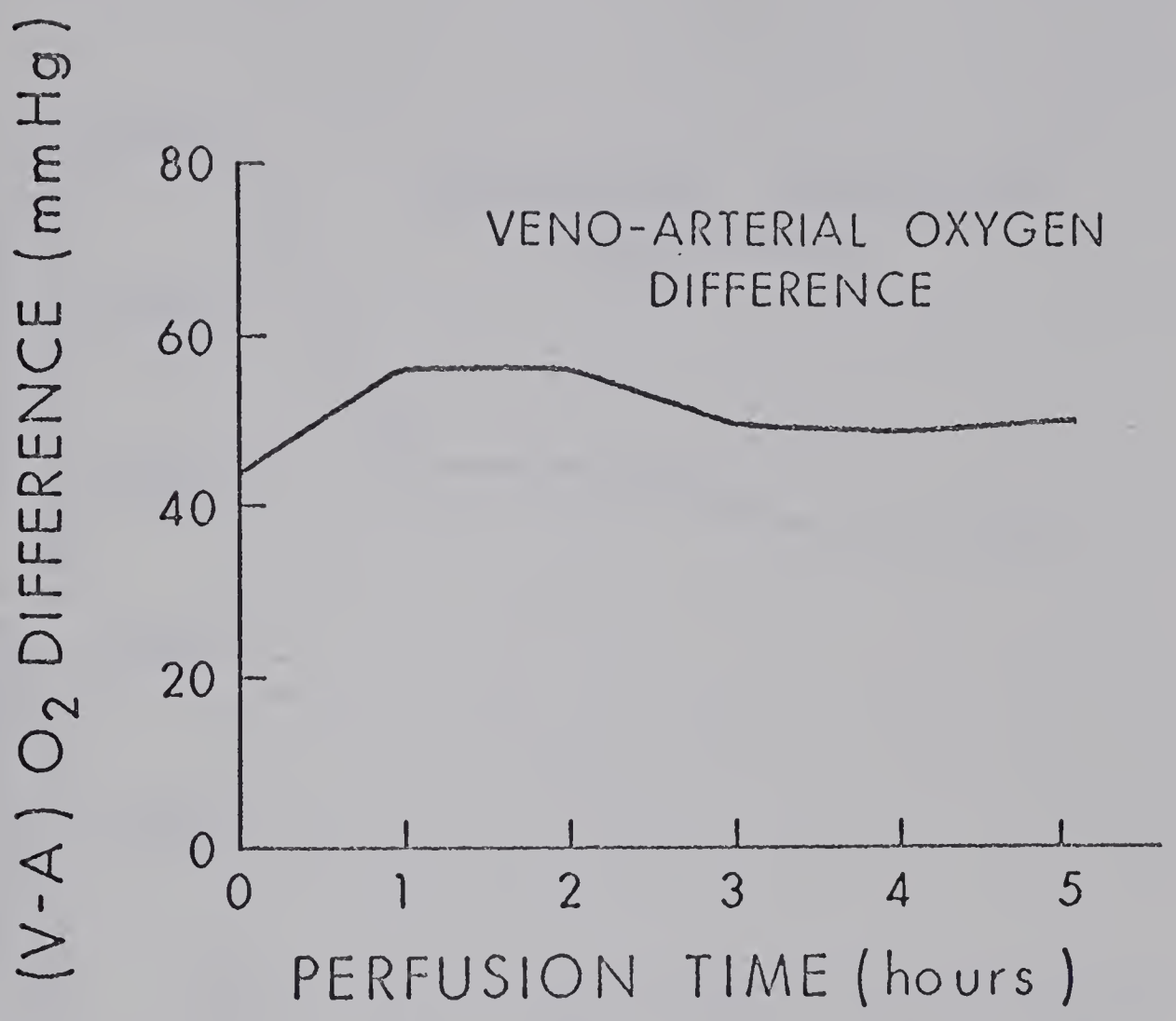


Fig. 24. Venoarterial oxygen differences in five hours unsupported perfusion.

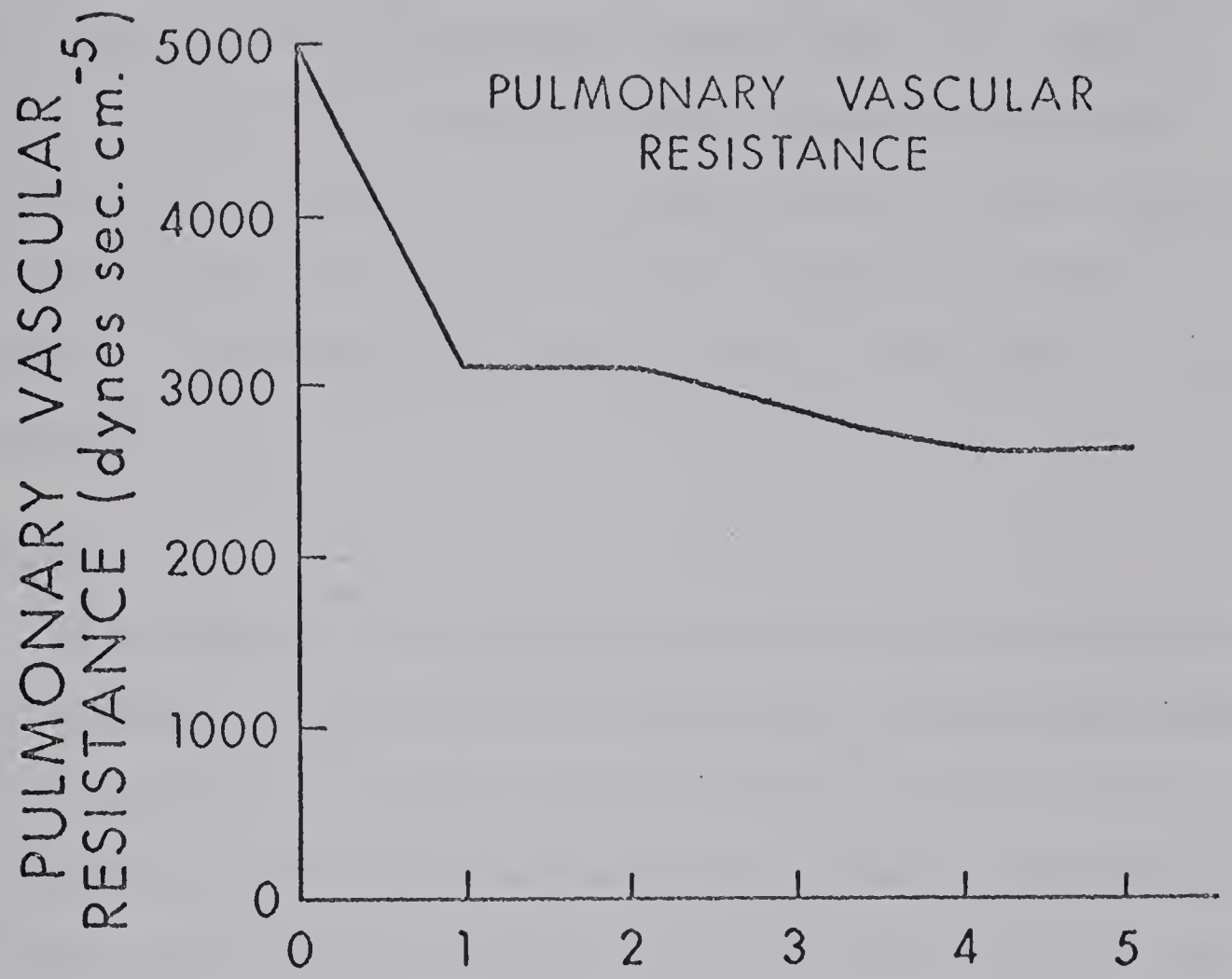


Fig. 25. Pulmonary vascular resistance changes during five hours unsupported perfusion.

by a high venoarterial oxygen gradient across the lobe remained adequate after five hours of perfusion (Fig. 24). Most interesting, however, is the fact that pulmonary vascular resistance did not rise over the experiments, and conversely declined (Fig. 25). This is contrary to observations in which a control animal was kept in the perfusion circuit (Chapter IV) for a comparable time period. The mean weight gain in these lobes after five hours perfusion was 45% in comparison to a 31% mean weight gain in supported lobes after 12 hours of perfusion.

Discussion

It is evident that metabolism proceeds in the isolated perfusion system. Glucose is very quickly utilized so that the glycolytic products lactate and pyruvate, accumulate both in the tissue and perfusate. Blood perfusate alone will metabolize glucose; however, accumulation of both tissue and perfusate lactate and pyruvate implies that the most significant activity is associated with the perfused pulmonary tissue. This is in agreement with Weber and colleagues[109] who found that the plasma perfused canine lung can utilize carbohydrate almost exclusively for its energy requirements and will produce lactic acid even when the lactic acid concentration in the perfusate reaches values greater than 100 mgm%. Although the liver, muscle masses, the heart, and possibly other organs, can remove lactate from the blood, little is known of isolated lung function in this respect. The fact that tissue lactate and pyruvate concentrations are elevated may be significant in that there is a preponderance of lactate or "lactate excess" characteristically associated with anerobic metabolism.[110]

With removal of glucose and other nutrients, the amount of energy available to the cell may be severely limited. Energy in the form of ATP or creatine phophate is critically important in maintaining cellular integrity. Subcellular structure plays a protective role in isolating essential cellular constituents, such as proteases and hydrolases (lysosomes) which are potentially destructive.[111] In addition, subcellular structure figures in biological energy transformations via the precise structure-bound mitochondrial systems of electron transport and oxidative phosphorylation,[112] the activities of which may have an influence on active transport and maintenance of ionic gradients.

The release of histamine and serotonin into the perfusion circuit is potentially significant. Histamine is concentrated in lung tissue mast cells and is present in concentration second only to that of the central nervous system.[113] Histamine has been shown to be released by hypothermic plasma perfusion of the lung by Brownlee[66] and is not metabolized by the isolated canine lung.[57] Histamine release may be due to anoxia, tissue trauma, emboli or deficient cellular energy. Serotonin is similarly ubiquitous in distribution, but is metabolized to some extent by the isolated canine lung.[57] Both substances have been termed 'permeability factors'[114] and can cause capillary damage. This could conceivably lead to stasis, capillary blockage and edema with organ weight gain and functional deterioration. As well, both substances stimulate contraction of smooth muscle. Experimentally histamine elevates pulmonary arterial and venous pressure without significant change in pulmonary vascular resistance;[115] it also induces bronchoconstriction with decreased vital capacity.[116]

Similar changes have been noted with serotonin[57] although precise mechanisms are controversial due to the fact that many effects are reflexly mediated and hence subject to influences such as pattern of innervation and spontaneous tone.

In the light of known histamine and serotonin release from the isolated perfused lung, it is interesting to note that the pulmonary vascular resistance in these experiments was actually lower than in a comparable period of the 12 hour perfusions (Chapter V) incorporating a donor animal. One could speculate that a substance which actually lowers pulmonary vascular resistance (e.g. bradykinin[117]) is being released into the perfusate of these experiments and is not being metabolized by a support animal. The presence of increasing vasoactive material in the perfusate was confirmed on isolated guinea pig ileum.* There are many vasoactive materials present in pulmonary tissue[118] and this aspect of perfusion deserves further study. The relevance to clinical states of extracorporeal circulation is immediate.

To counteract the observed metabolic and pharmacologic changes in blood perfusion of canine lung, a very large volume of perfusate could be implemented both to provide greater nutrient stores and to dilute and minimize vasoactive substances. Another approach might be to deplete the lung of histamine before perfusion with compounds such as 48/80[119] or to add antihistamines and extra nutrients during perfusion. Similarly with serotonin, depletion of the donor animal could be accomplished with prior resperine treatment or serotonin antagonists

---

* Appendix IV

such as d-lysergic acid[120] might be added to the perfusion system.

Conclusions

1. Isolated unsupported normothermic blood perfusion of the canine lung rapidly removes perfusate glucose with production of metabolic acids.
2. Vasoactive materials are released from the perfused canine lung.
3. Further work is required to elucidate the etiology, variety and significance of these vasoactive materials.

CHAPTER VI

POSITIVE VERSUS NEGATIVE PRESSURE VENTILATION

Ventilating isolated pulmonary lobes with intermittent negative pressure would intuitively seem to provide better functional support in isolated perfusion than unphysiologic positive pressure ventilation. In spite of this, many investigators [59,60,68,75] continue to implement positive pressure ventilation in lung perfusion and some workers have even maintained that it is necessary to defer the formation of edema fluid.[43,75,121,122] Although clinically assisted ventilation with positive pressure has found considerable use in the treatment of pulmonary edema and respiratory insufficiency of varied causes, it has been associated with a variety of functional derangements. Nash et al.[123] have found increased dead space in patients treated with positive ventilatory assistance and other workers have found that, although gas distribution is relatively unchanged, pulmonary blood flow is shifted away from well ventilated areas with significant increase in ventilation-perfusion disproportion during prolonged assistance.[124] The increased alveolar and intrathoracic pressure during positive pressure ventilation reduces venous return, hence the salutory effect in pulmonary edema.[125]

Although the changes resulting from positive pressure ventilation have been studied extensively, experimental techniques have varied widely.[126-128] Clinical studies have been obscured by the many variables necessarily associated with patient care. Because of the differing opinion regarding the use of positive and negative pressure ventilation in isolated lung perfusion, it was decided to evaluate the relative merits of both under carefully controlled conditions.

Methods

Eight healthy mongrel dogs weighing from 15 to 32 kgm were used in this study. Left lower pulmonary lobes were removed in the usual fashion and were perfused in a circuit incorporating the donor animal (Fig. 2). Perfusion was initiated in the usual fashion with blood flow at normothermic conditions gradually increased to a constant 15 cc/donor kgm/minute. The perfusion circuit was allowed to stablize with negative pressure ventilation for 30 minutes and then alternate 30 minute periods of negative and positive pressure ventilation were carried out. In each case, venous pressure was accurately maintained at mean 2 mm Hg pressure. Ventilation with intermittent negative pressure was set at -9/-4 cm HOH pressure excusions at a rate of 12/minute. After a 30 minute period of intermittent negative pressure ventilation lobe tidal volume was accurately measured with a Douglas bag collection of 10 breaths measured on a spirometer. Following this, lobe stretch or functional residual capacity was maintained with a constant chamber negative pressure of -4 cm HOH but a tidal volume equal to that measured under negative pressure was imposed upon the lobe by means of a positive pressure respirator*¶ fitted to the bronchial cannula. The situation is somewhat analogous to positive pressure respiration in the closed chest animal. In this manner the functional residual capacity or lobe stretch, flow, venous pressure and tidal volume were kept constant under alternating negative and positive pressure ventilation at 30 minute intervals.

---

* Harvard Respiratory Pump, Special Product #1170, Harvard Apparatus Co. Inc., Dover, Mass.

¶ Respirator phase adjustment - Inspiration:Expiration = 1:2 in both negative and positive pressure ventilation trials.

Measurements

Pulmonary arterial and venous pressures were monitored continuously and were recorded after 30 minutes of negative or positive pressure ventilation, at which time pulmonary arterial and venous blood gases were also determined. Lobe tidal volumes and ventilation pressures, either positive or negative were recorded at the same time intervals. From this data and the set flow rates and venous pressure, compliance and pulmonary vascular resistance were determined in the usual fashion. The weight of each lobe was monitored continuously.

Pulmonary blood volume changes were determined in each case with the aid of dye dilution technics.[129] One milligram Cardio-Green dye was injected into the pulmonary artery from a fixed injection site in each experiment and the blood-dye mixture in the pulmonary venous blood was drawn through a densitometer* at a fixed rate of 50 cc/minute. A dye dilution curve with automatic determination of curve area is thus produced. Knowing time of injection and the time taken for dye to traverse the tubing involved in each case, the mean transit time across the perfused lung can be easily calculated (see Appendix VI). It has been shown that blood volume equals mean transit time X flow, and flow in this case is known. The appearance time in each case was carefully measured with a stopwatch - the time from dye injection to dye appearance on the dye dilution curve represents the time taken for the dye to traverse the tubing involved (constant) and the time taken to traverse the perfused lobe to determine whether positive or negative inflation influenced particle transit across the lobe at constant mean flow.

---

* Beckman Cardio-Densitometer, Beckman Instruments, Palo Alto, California.

Results*¶

In Table VIII mean function and hemodynamic changes are reported for eight lobes subjected to four different periods of alternate negative and positive pressure ventilation. In Table IX the functional differences between negative and positive pressure ventilation at each of the four trials are presented and the cumulative mean differences with standard deviations have been calculated. Students' t test has been applied to the calculated differences in function for the pooled results (Appendix XI) and significance determined. Figures 26-29 reflect the data from the above tables in easily visualized form.

It is seen that under equal conditions of flow, venous pressure and lobe stretch (functional residual capacity) that equal volume ventilation with positive pressure in each trial results in considerably greater vascular resistance across the perfused lobe and that, moreover, this gradient tends to increase with time. It is evident that this is due to elevation in pulmonary artery pressure.§ Pooled results indicate that this rise in pulmonary vascular resistance is very significant. In opposite fashion, the mean compliance of the lobes is diminished with positive ventilation and the gradient is again significant. Gas exchange is significantly less effective for both oxygen and carbon dioxide under conditions of positive pressure ventilation (Fig. 21). This was noted during the experiments as a noticeable darkening of

---

* Sample values and pulmonary vascular resistance determinations are recorded in Appendices VII-IX.

¶ Individual dynamic compliance rations (tidal volume/ventilating pressure) are recorded in Appendix X.

§ Since $PVR = \frac{PA - PV}{Flow}$ and PV and Flow are constant.

TABLE VIII - MEAN FUNCTION AND HEMODYNAMICS OF EIGHT LOBES PERFUSED WITH ALTERNATING NEGATIVE AND POSITIVE PRESSURE VENTILATION AT EQUAL TIDAL VOLUMES

	Pulmonary Vascular Resistance (dynes sec. cm.$^{-5}$)		Lobe Compliance (cc./cm. HOH/ lobe g.) x 100		(A-V) CO_2 Difference (mm. Hg)		(V-A) O_2 Difference (mm. Hg)		Dye Appearance Time (seconds)		Pulmonary Blood Volume (cc.)	
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Hour 1 (Trial 1)	3380 ±1700	3630 ±1550	35.2 ± 7.3	23.6 ± 3.4	15.4 ±3.7	10.1 ±1.9	62.0 ±23.6	51.5 ±18.2	8.7 ±1.7	8.6 ±1.4	44 ±16	45 ±15
Hour 2 (Trial 2)	3150 ±1280	3500 ±1230	31.4 ± 8.1	22.0 ± 2.9	14.1 ±5.0	11.4 ±4.0	60.9 ±16.0	48.9 ±17.8	9.1 ±1.2	8.6 ±1.4	48 ±11	47 ±10
Hour 3 (Trial 3)	3440 ±1100	4200 ±1200	30.0 ± 5.1	21.5 ± 2.4	15.0 ±4.7	13.0 ±5.6	60.1 ±16.2	45.6 ±17.1	8.9 ±1.3	7.8 ±0.8	46 ±11	42 ±11
Hour 4 (Trial 4)	4000 ±1380	4940 ±1410	27.9 ± 6.6	19.2 ± 3.4	14.4 ±4.5	10.0 ±6.7	45.8 ±18.1	37.5 ±15.0	8.0 ±1.0	7.4 ±0.9	42 ±15	37 ±13

TABLE IX - MEAN DIFFERENCES IN FUNCTION AND HEMODYNAMICS BETWEEN NEGATIVE AND POSITIVE PRESSURE VENTILATION IN EIGHT LOBES

	Pulmonary Vascular Resistance (dynes sec. $cm.^{-5}$)	Lobe Compliance (cc./cm. HOH/ lobe g.) x 100	(A-V) CO_2 Difference (mm. Hg)	(V-A) O_2 Gradient (mm. Hg)	Dye Appearance Time (seconds)	Pulmonary Blood Volume (cc.)
Hour 1 (Trial 1)	250 ±350	-11.6 ±8.1	-5.2 ±3.7	-9.5 ±11.3	-0.1 ±0.6	0.5 ±3.9
Hour 2 (Trial 2)	350 ±510	-9.4 ±10.2	-3.0 ±2.8	-12.0 ±9.3	-0.6 ±0.5	-0.9 ±7.1
Hour 3 (Trial 3)	760 ±450	-8.3 ±5.5	-2.0 ±3.7	-13.3 ±9.4	-1.1 ±0.7	-4.3 ±2.5
Hour 4 (Trial 4)	930 ±370	-8.5 ±8.1	-4.6 ±4.1	-8.3 ±10.5	-0.7 ±0.8	-5.1 ±3.4
Cummulative Mean Difference of 4 x 8 = 32 values	570 ±370	-9.5 ±8.0	-3.7 ±3.7	-10.8 ±9.9	-0.6 ±0.7	-2.5 ±4.9
T value	4.49	4.71	3.94	4.30	3.38	2.01
Significance	p<.005	p<.005	p<.005	p<.005	p<.005	p<.05

+ = increased with PPV

- = decreased with PPV

Fig. 26A.

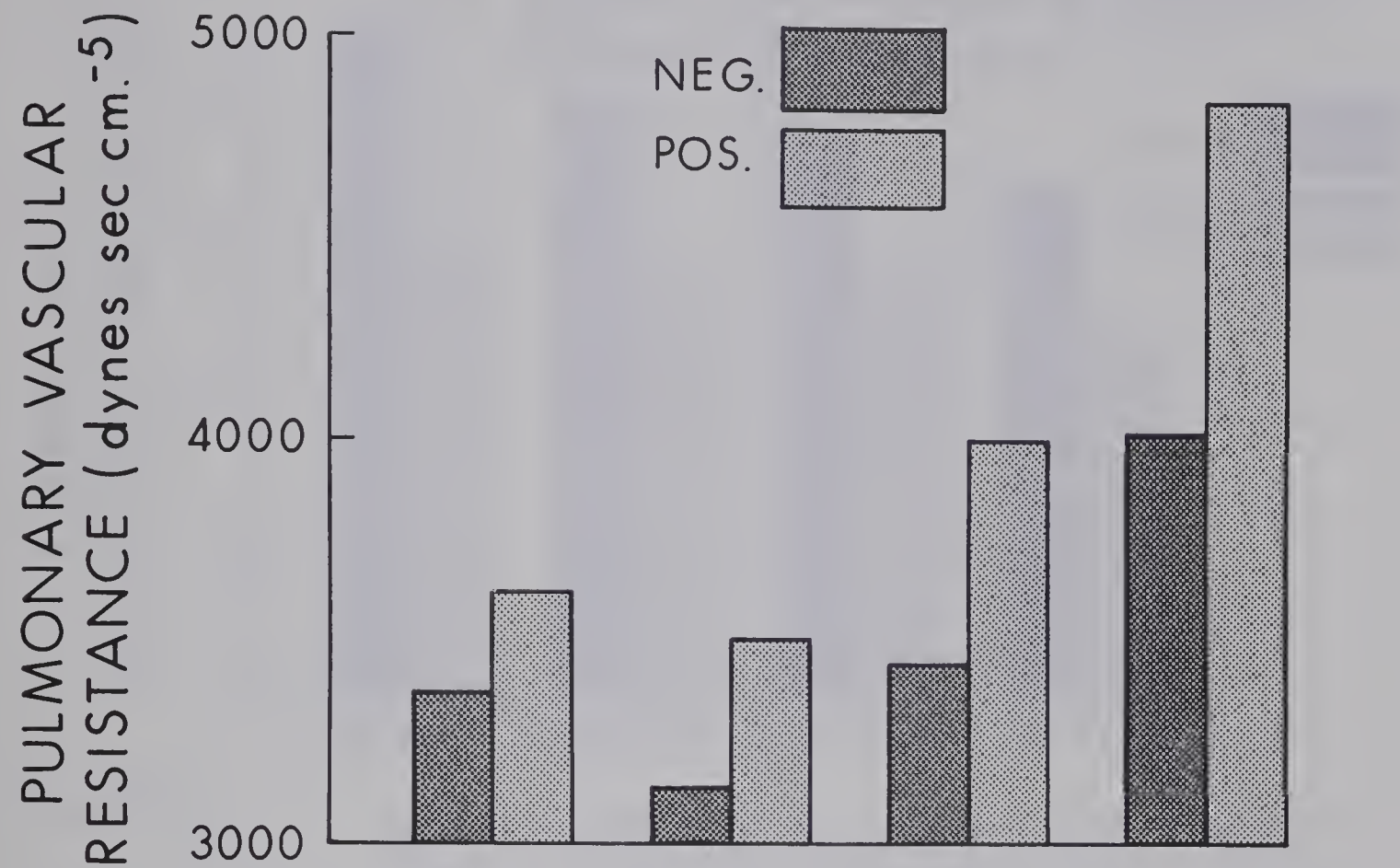


Fig. 26B.

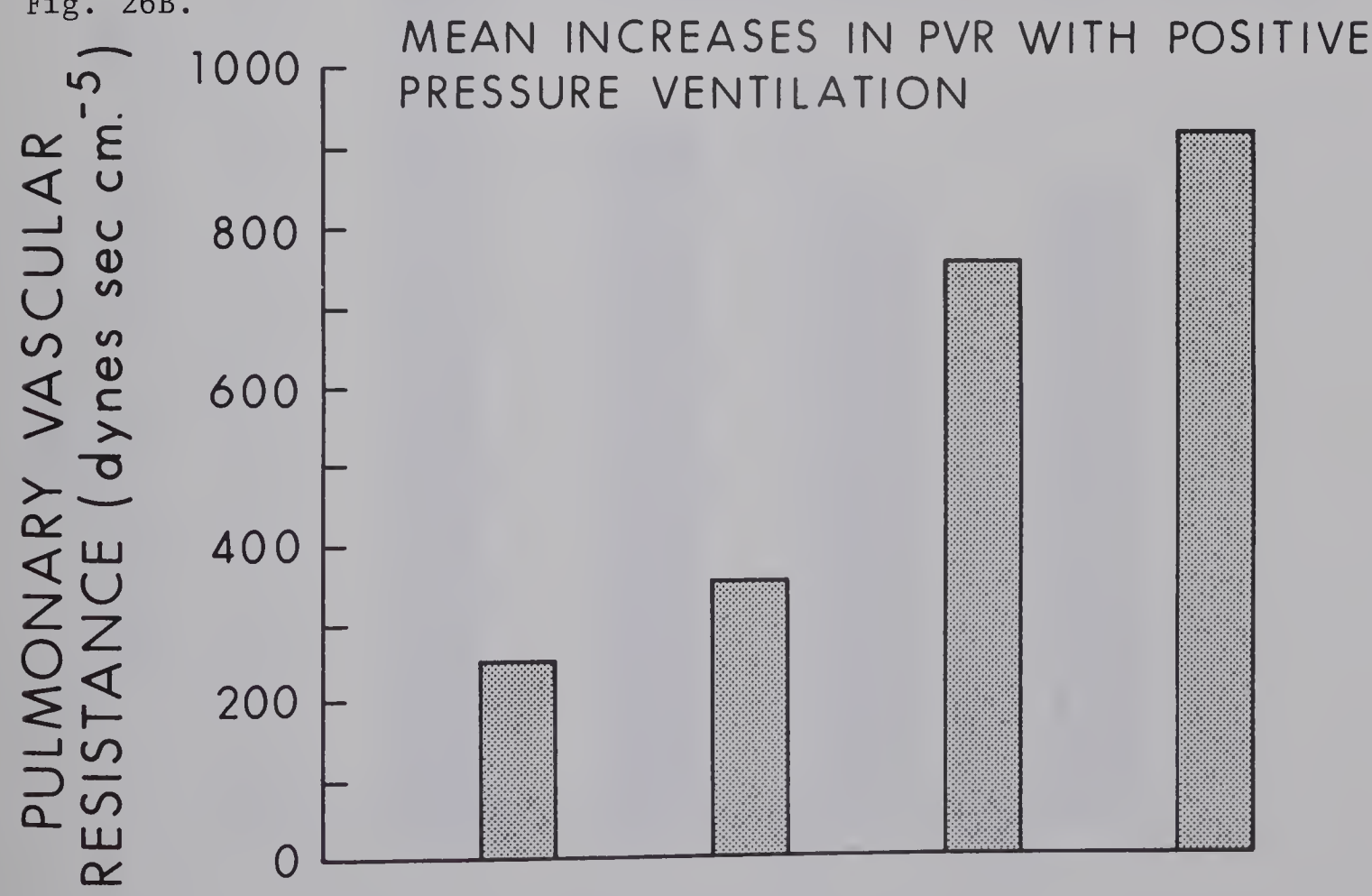


Fig. 27A.

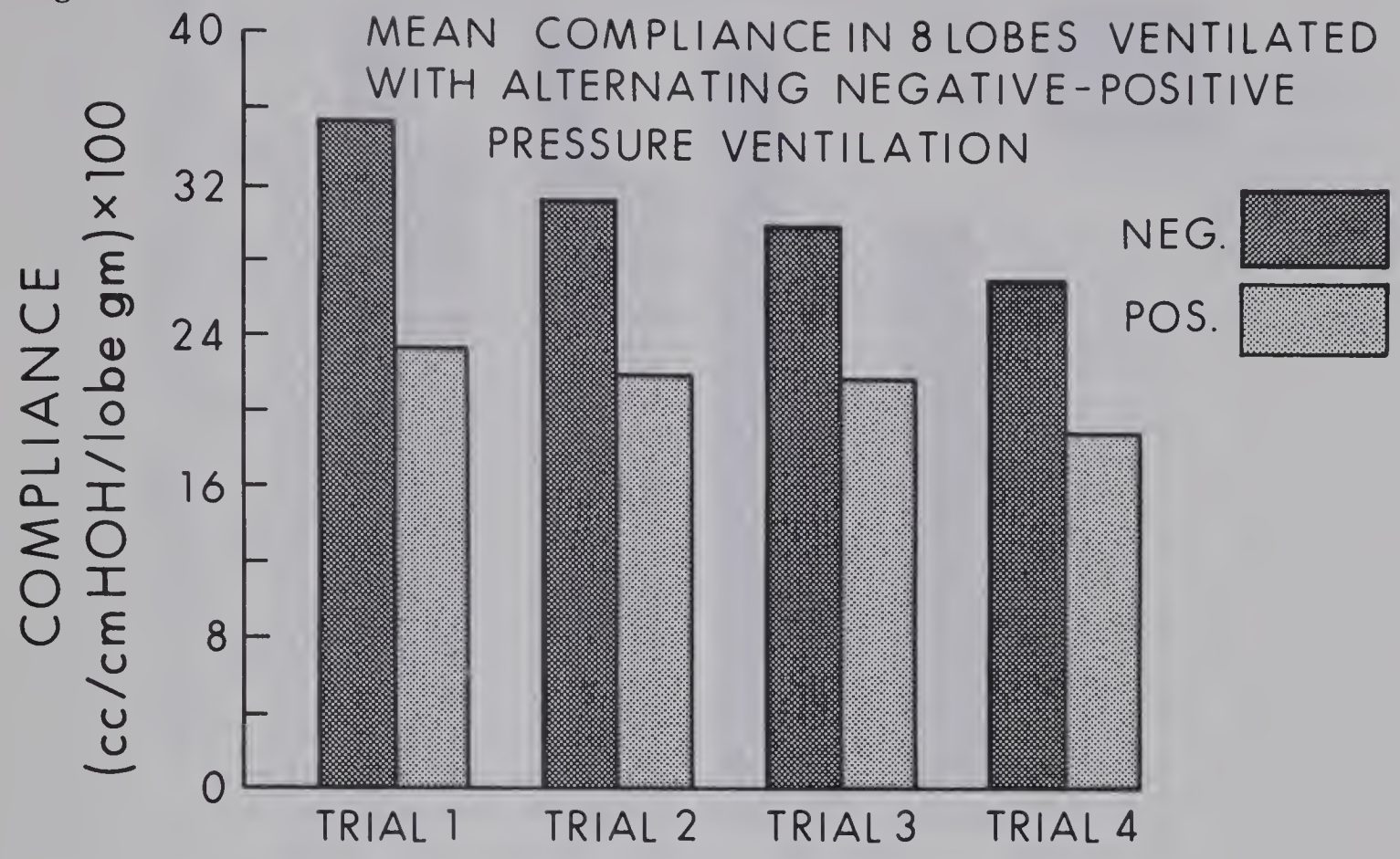


Fig. 27B.

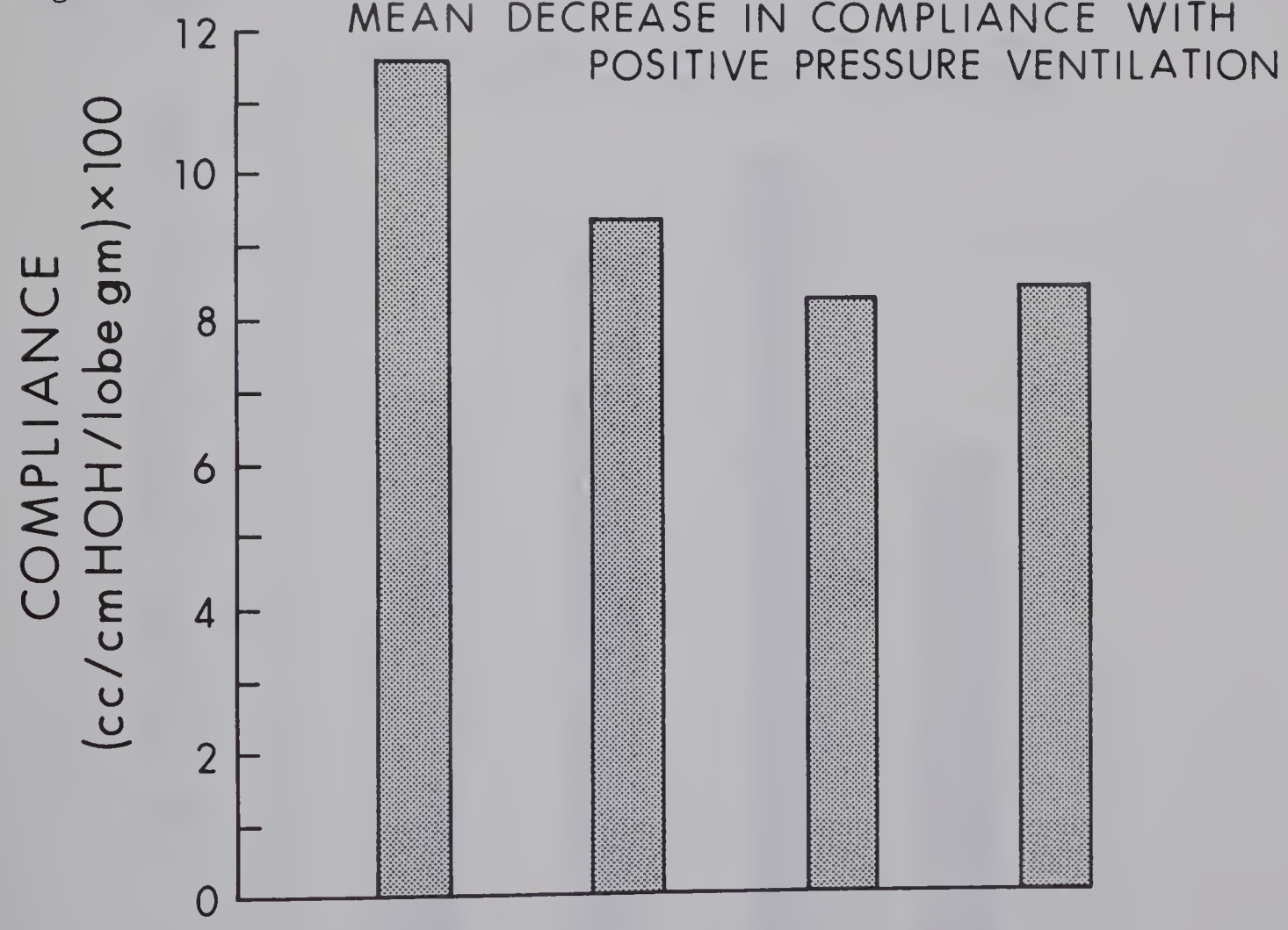


Fig. 28A.

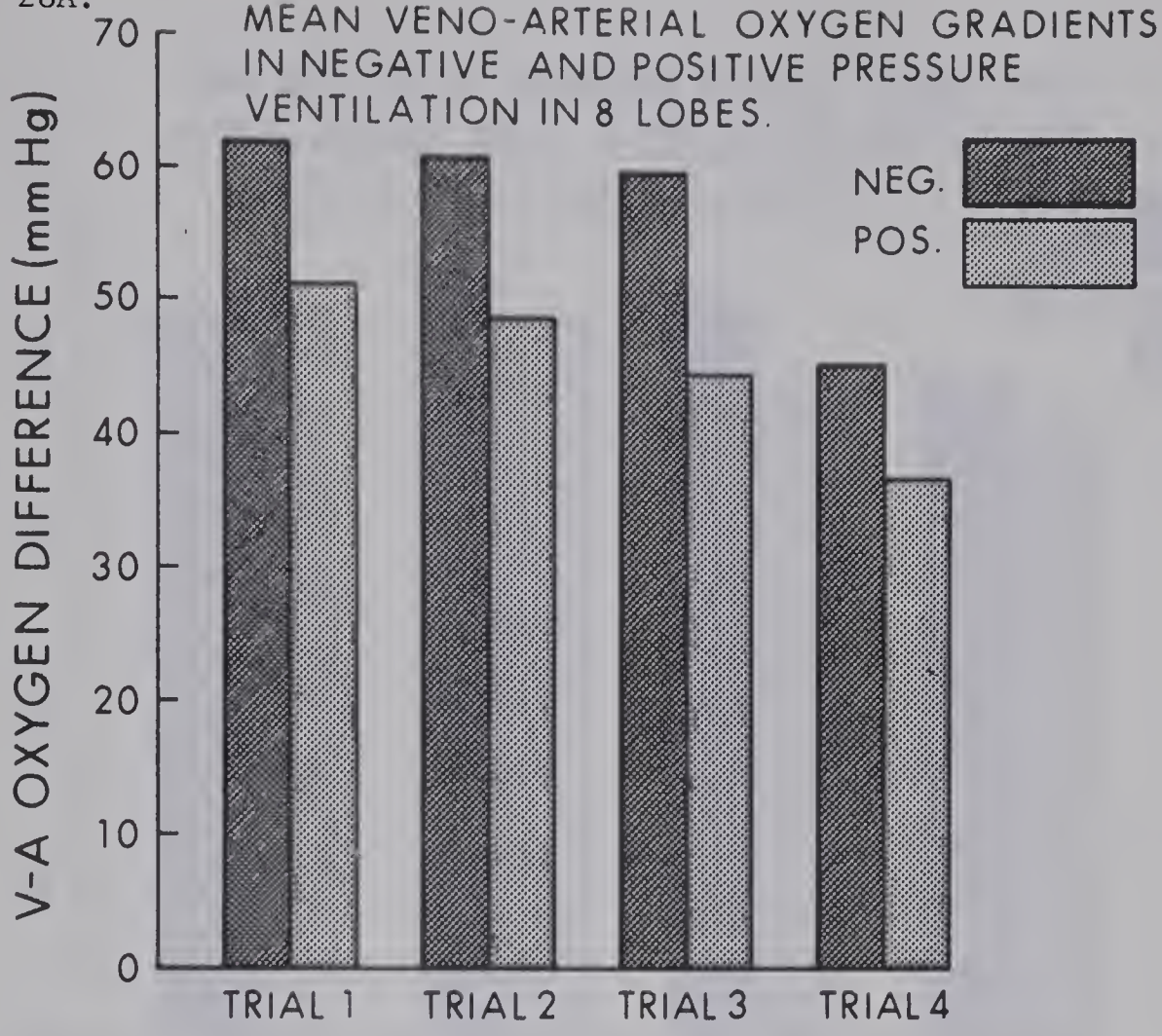


Fig. 28B.

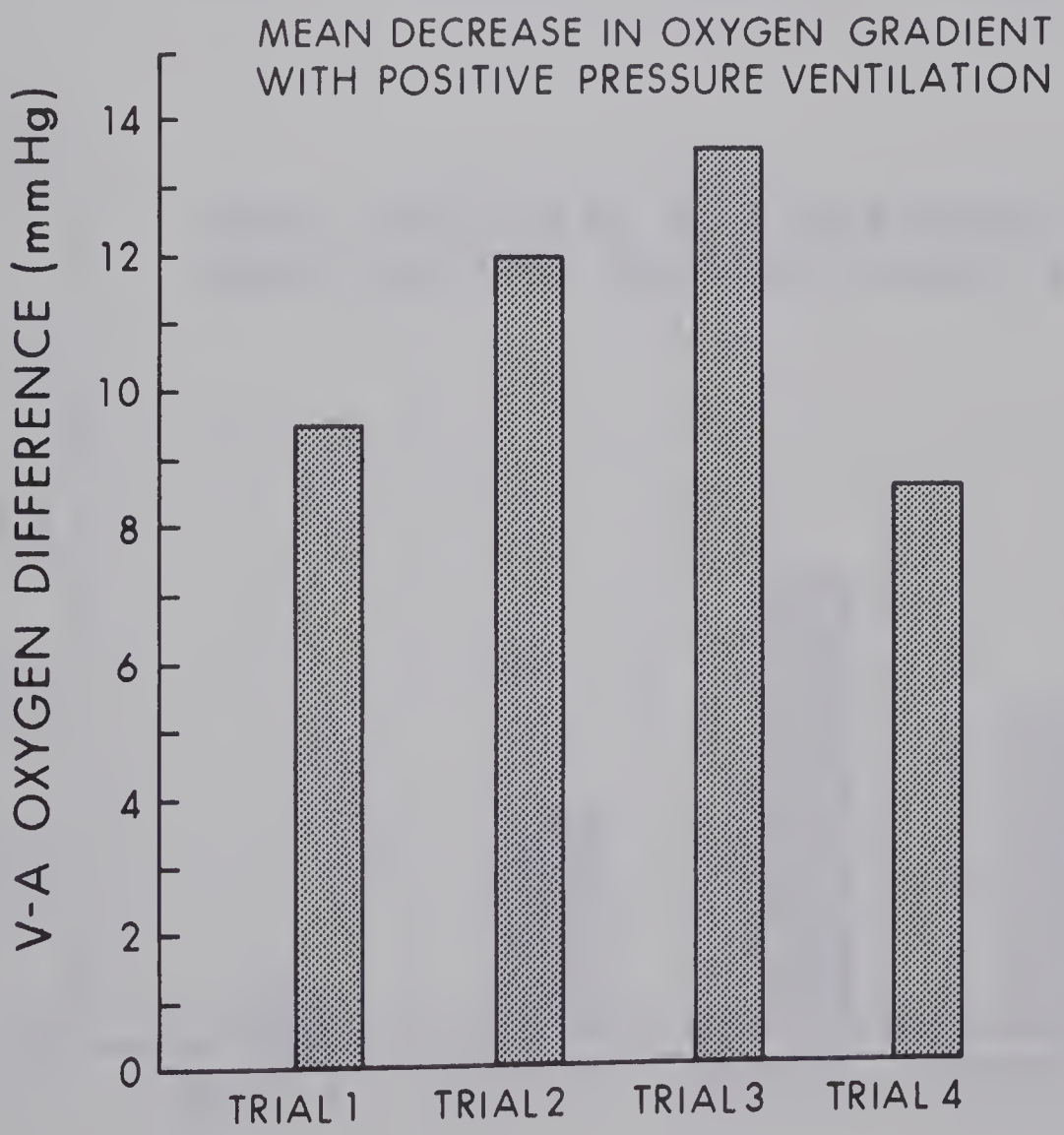


Fig. 29A.

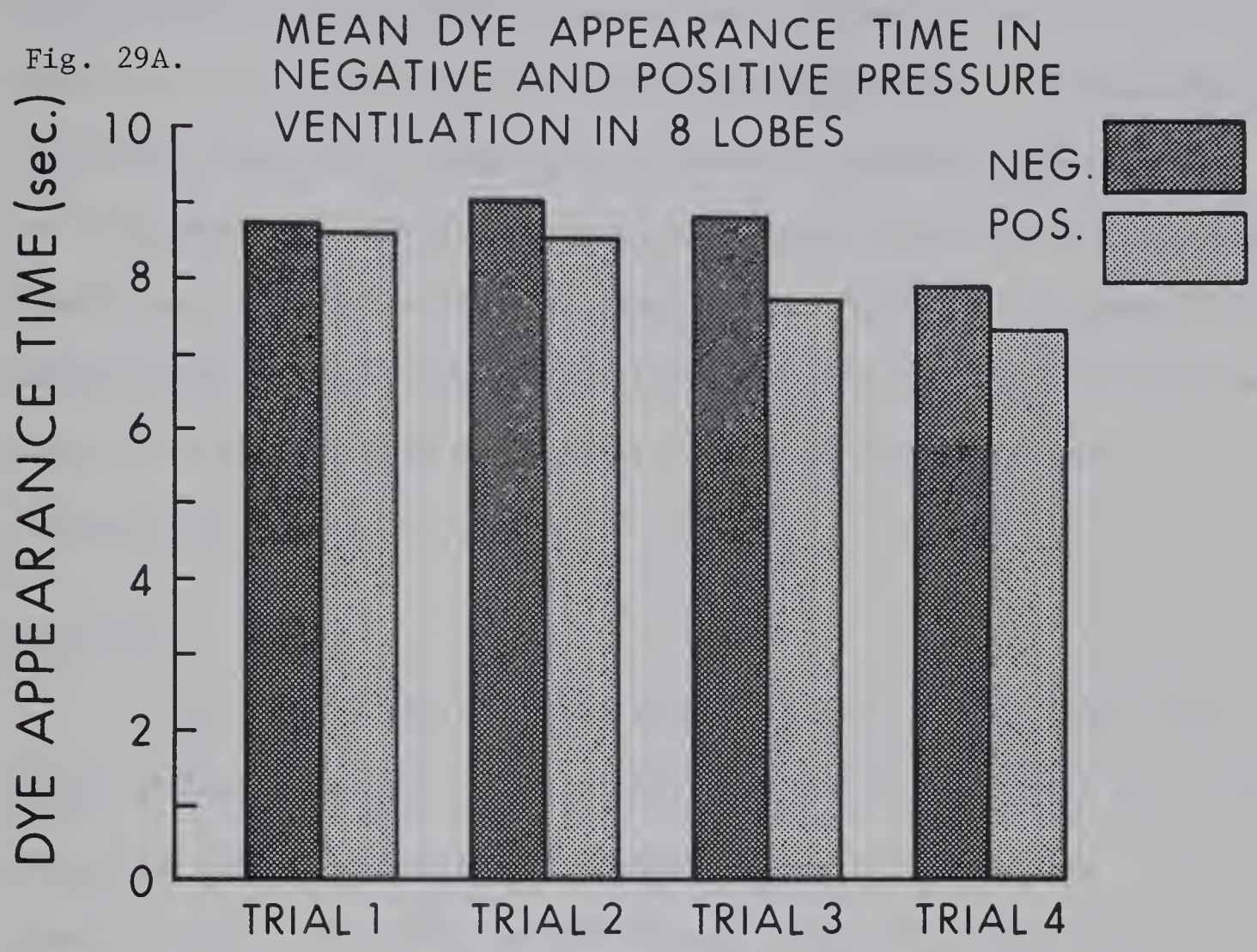
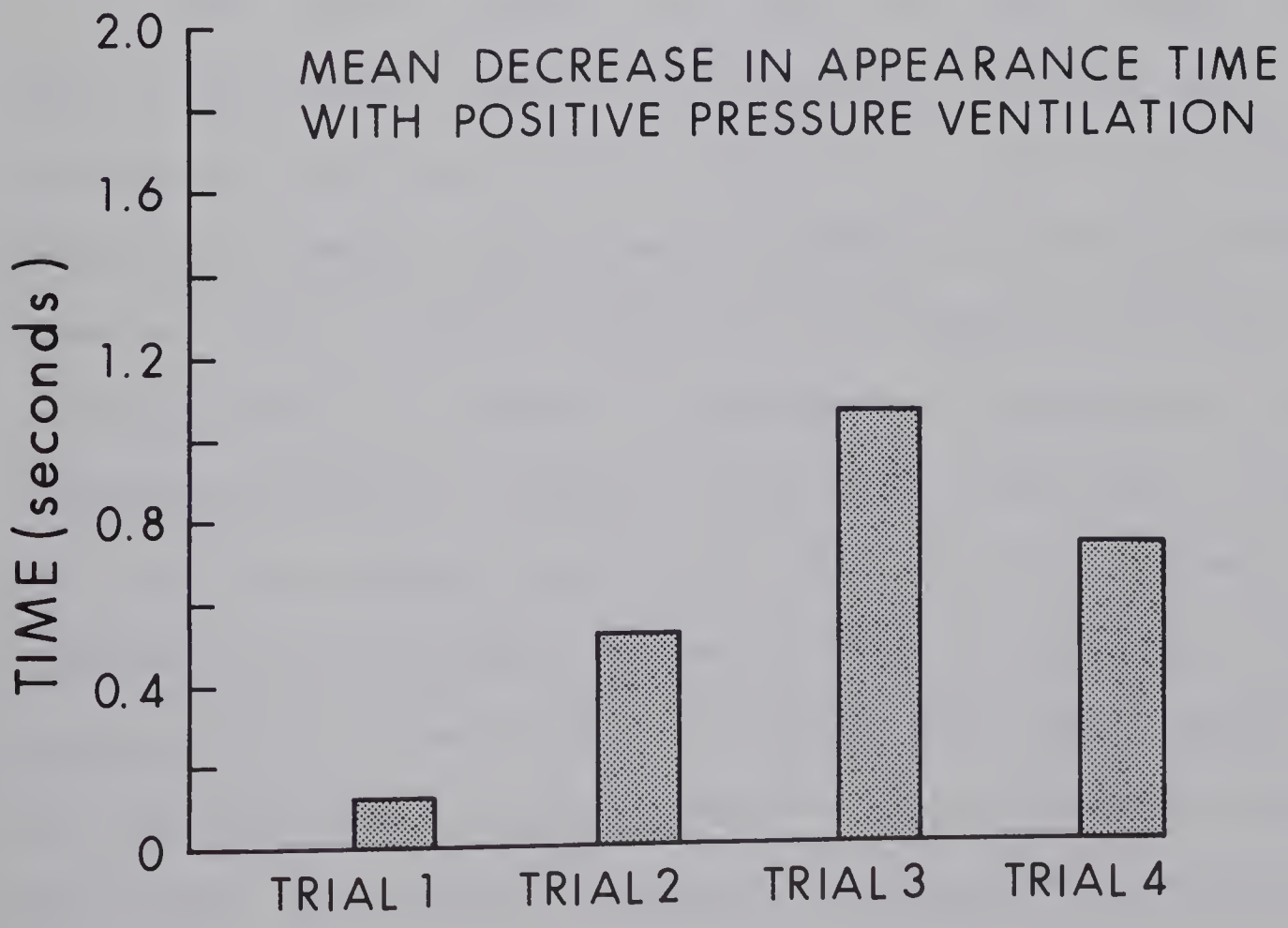


Fig. 29B.



arterialized blood from the pulmonary vein. That this might be due to abnormal circulatory shunts was investigated with aid of dye dilution curves and dye transit (Fig. 22) through the perfused lobe was found to be consistently slightly more rapid through the perfused lobe under conditions of positive pressure ventilation. Although pulmonary blood volume tended to diminish with positive pressure ventilation, in later trials the results were not considered consistent enough to be significant.

Discussion

The results reported here show that under dynamic conditions of isolated lobe perfusion and carefully controlled physiologic flow, lobe expansion and venous pressure, positive pressure ventilation of the lung is associated with elevated pulmonary vascular resistance, diminished compliance and less effective gas exchange.

With negative pressure ventilation, the lung parenchyma responds first to any pressure change, and the expanding parenchyma exerts traction upon the blood vessels, tending to open them and facilitate blood flow. However, with inflation secondary to increasing endotracheal pressure, there is initial compression of the smaller thin walled pulmonary vessels as a result of overexpansion of the airways. Positive pressure ventilation in isolated lobe perfusion thus tends to reduce the size of the pulmonary vascular bed effective in gas exchange; with constant flow as implemented in these conditions, circulation is then preferentially diverted through those larger thick walled vessels which are less responsive to pressure gradients. This explanation accounts for the more rapid transit of indicator dye through the perfused lobe

under conditions of positive pressure ventilation.

It is recognized that ventilation of the isolated lobe in these experiments is not strictly comparable to clinical positive pressure ventilation; the chest wall has its own compliance in clinical situations, whereas ventilation in these experiments took place within a rigid and unyielding chamber. Recent clinical workers[123,130] have been impressed with the development of progressive deterioration in certain patients requiring mechanical artificial ventilation, but have attributed this to the known toxic effects of high oxygen concentrations. Arterial hypoxemia has, however, been noted in patients treated with room air positive pressure ventilation[131] and this may be explainable in terms of the above experimental model. Several clinicians[132,133] have found treatment of the respiratory distress syndrome of infants with a whole body negative pressure respirator much more effective than prior positive pressure assistance.

In terms of isolated lung perfusion, it is clear that positive pressure ventilation is undesirable. Gross edema is not produced over four hours perfusion with either means of ventilation and the small drop in lobe weight with positive pressure respiration is attributable to a reduction in the effective microcirculation. The consequent elevated pulmonary artery pressure and pulmonary vascular resistance impose unnecessary pressures on pulmonary tissue and their circulatory effects disturb nutrient supply and promote hypoxia. The observed arteriovenous shunt probably results in anoxia distal to the shunts with consequent capillary damage.

Summary and Conclusions

1. Equal volume ventilation under controlled conditions of flow, venous pressure and functional residual capacity was compared in eight isolated perfusion experiments.
2. Positive pressure ventilation consistently produced significant elevation in pulmonary vascular resistance, diminished compliance and impaired gas exchange.
3. Positive pressure ventilation in the described experimental model compromises the normal pulmonary circulation and is not indicated in perfusion preservation of the isolated canine lung.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Several aspects of functional preservation of canine lungs have been investigated with the aid of a versatile organ perfusion apparatus:

1. Hypothermic hyperbaric 24 hour storage is not effective in preserving normal function in canine pulmonary lobes. Lobes stored in this manner deteriorate rapidly with development of gross edema within 90 minutes of perfusion. Non-stored control lobes continue to function adequately without edema formation after 4 to 5 hours of perfusion. Isolated organ perfusion is a valuable means of assessing both graft function and the efficacy of storage techniques.

2. Careful simulation of normal intrathoracic ventilation and hemodynamics and maintenance of normal perfusate composition by incorporating a donor animal into the perfusion circuitry will permit routine preservation of ex vivo lobe function and morphology for periods in excess of 12 hours.

3. In isolated lobe perfusion excluding a support animal, perfusate composition quickly changes. Glucose is rapidly metabolized; lactic and pyruvic acid accumulate and promote metabolic acidosis. Vasoactive substances, including histamine and serotonin, are released during isolated lung perfusion and may significantly damage the perfused organ. Further work is indicated to determine whether addition of nutrients during perfusion will support lobe function for

longer time periods. Similar work is required to elucidate the variety and significance of vasoactive materials

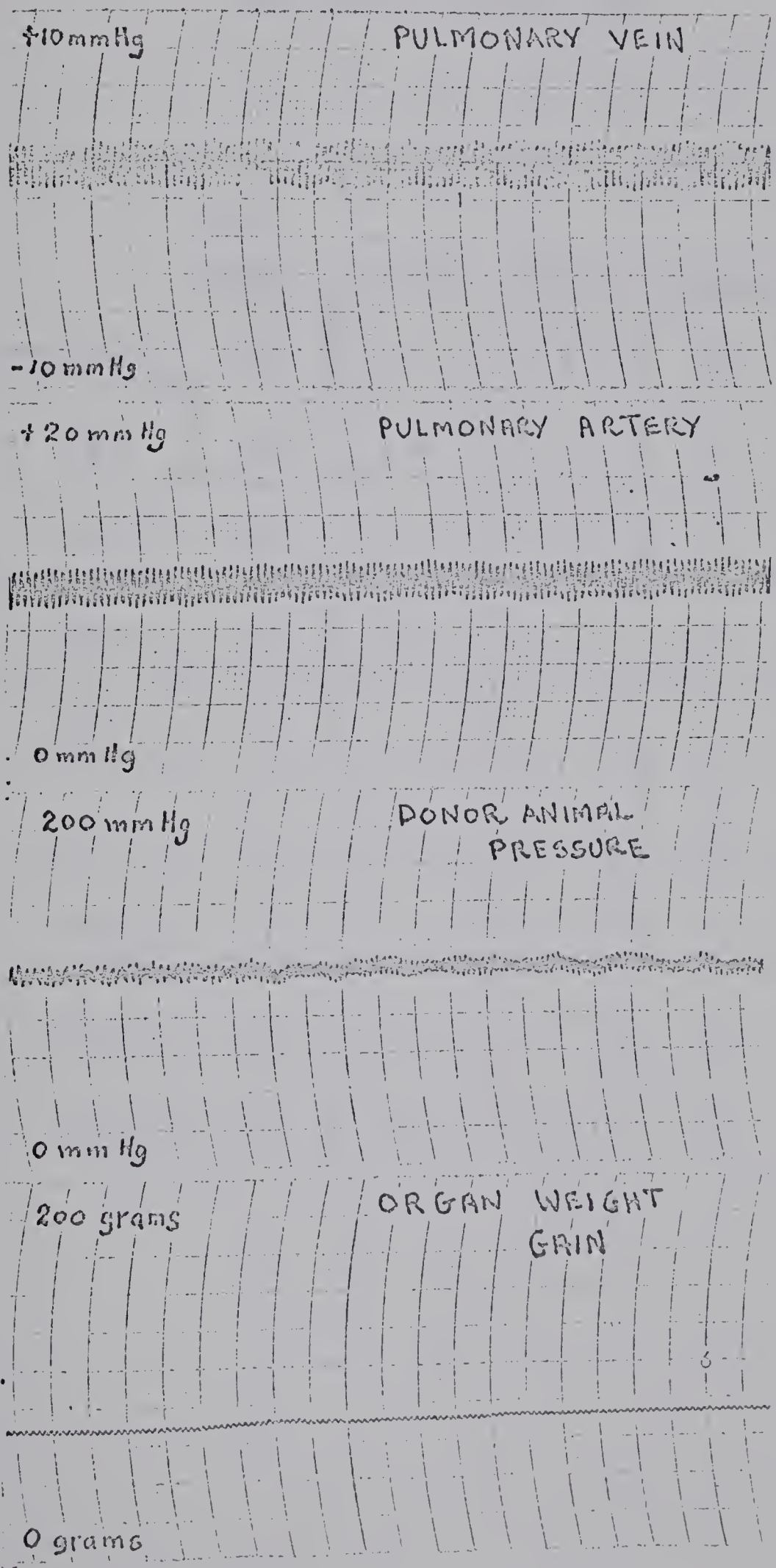
4. Evaluation of positive and negative pressure ventilation in isolated lung perfusion indicates that positive pressure ventilation is associated with increased pulmonary vascular resistance, diminished compliance and shunting. Hypoxia, abnormal pressure gradients and diminished flow at the microcirculatory level indicate that positive pressure ventilation is not beneficial in isolated lung perfusion.

These studies commonly emphasize that preservation of the isolated canine lung requires strict replication of the normal physiology. The nutrient demands of each cell must be met and its metabolic wastes removed. Bacterial, embolic, osmotic and thermal trauma must be minimized and precisely normal circulation maintained. Perfusion technology is becoming increasingly sophisticated and the current developments in pulsatile pumps and non-clotting plastics will surely be associated with new success.

One limiting factor in organ preservation experiments is the lack of knowledge regarding the precise metabolic requirements of ex vivo organs. Further, very little is known about the effects of temperature, duration of perfusion or perfusate composition on isolated organ metabolism. With forseeable progress, organs may one day be maintained ex vivo for weeks, months or longer, in instant readiness for replacement in man.

Appendix I Fig. 30

Sample Dynograph Tracings



Appendix II Table X

Individual Venoarterial Oxygen Gradients During Perfusion of Nine Stored Pulmonary Lobes

	Time				
	0 min.	15 min.	30 min.	45 min.	60 min.
Expt. B-2	35	2	1	8	1
B-3	26	2	1	6	19
B-5	18	4	5	3	4
B-7	9	19	2	18	1
B-8	71	9	0	1	2
B-10	67	35	15	10	19
B-12	64	2	0	2	2
B-13	53	13	5	6	4
B-14	57	39	11	2	1

Appendix III Table XI

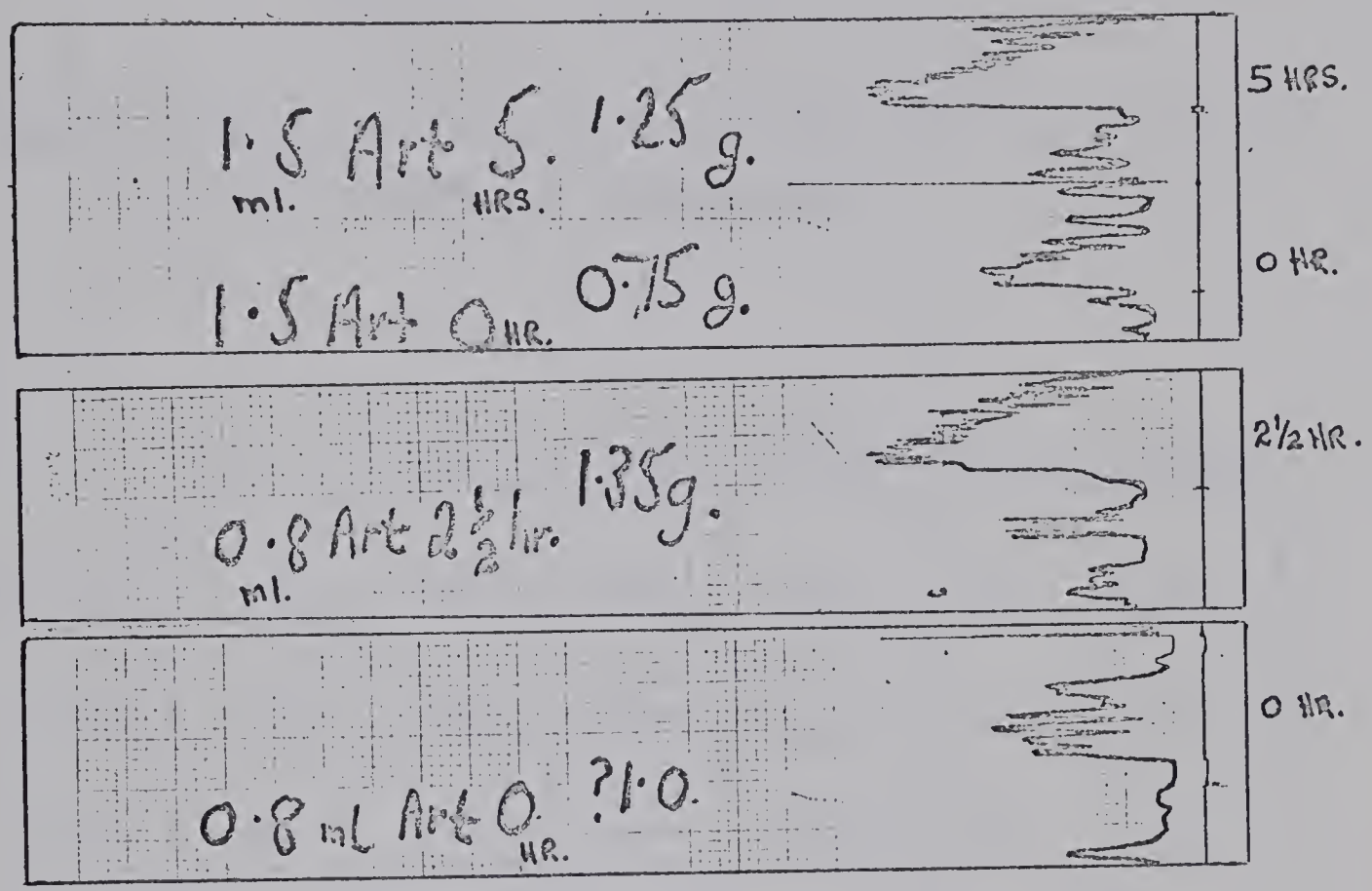
Individual Ratios of Tidal Volume (cc) to Ventilating Pressure (cm HOH) during 12 hour perfusions

Eight Pulmonary Lobes

	Perfusion Time (Hours)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Expt. G-1	100/5	135/6	110/6	110/6	93/4	100/6	70/6	70/5	60/6	50/6	50/6	35/6	45/6
G-3	130/6	140/5	145/6	120/6	110/6	110/6	85/6	95/6	95/6	85/5	115/4	145/5	125/6
G-4	75/5	140/6	125/6	120/6	110/6	105/6	110/6	115/6	105/6	105/6	85/7	100/6	75/6
G-5	200/5	195/5	200/6	185/5	155/4	155/4	190/5	200/6	190/6	215/6	190/6	185/6	175/7
G-6	80/7	65/6	55/7	85/7	70/7	50/6	60/6	60/6	85/6	80/6	70/6	65/6	55/6
G-8	65/5	60/5	50/5	180/5	80/5	75/5	70/5	65/5	55/6	50/5	50/5	50/5	50/5
G-9	90/5	115/7	105/6	165/4	135/5	120/5	100/5	100/5	95/5	95/5	90/5	90/5	90/6
G-10	155/6	120/5	120/5	105/5	105/5	95/5	105/5	105/6	95/6	135/6	80/7	125/6	115/6

Appendix IV Fig. 31

Vasoactive Materials in Lung Perfusate



Perfusate samples were evaluated for contractile effect on isolated guinea pig ileum.* The ileal segment was immersed in Tyrode's solution containing 2×10^{-8} gm/litre atropine and mepyramine. Contraction of the ileal segment was converted to grams tension as calibrated on a Sanborn recorder. This was only a pilot project and few samples were evaluated in this way but clearly greater contraction of the ileal segment was produced after 2 and 5 hours perfusion. This serves to confirm the release of vasoactive substances into the isolated lung perfusate and emphasizes their possible significance.

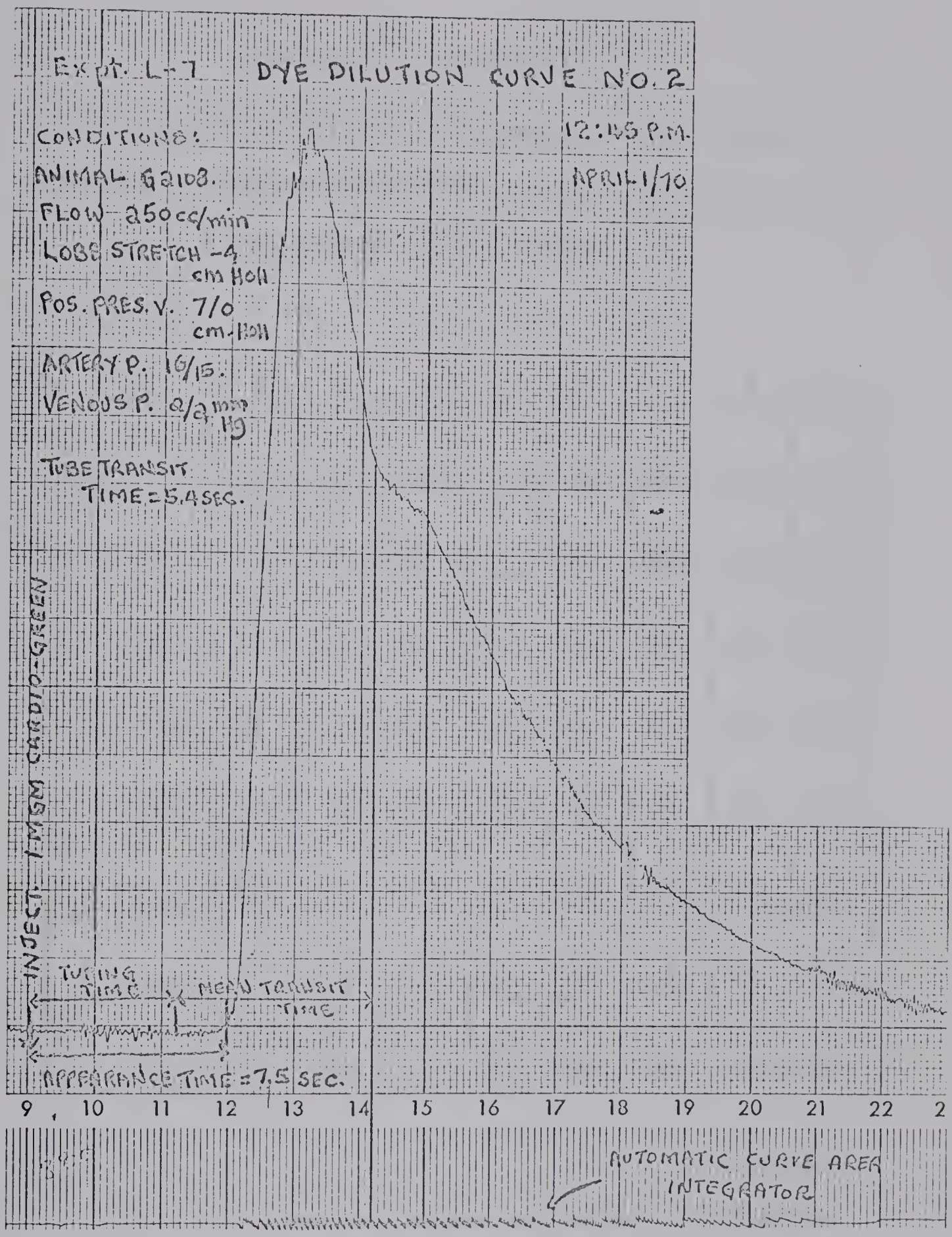
* Courtesy of Dr. Dave Tyler

Appendix V Table XII

Individual Blood Glucose Determinations During 5 Hours Recirculation Perfusion (Bubble Deoxygenation)

	0	1 hour	2 hours	3 hours	4 hours	5 hours
Expt. M-2	71	47	46	34	29	11
M-3	45	36	20	4	5	5
M-5	105	76	42	20	20	4
M-6	57	45	25	11	11	5
M-7	82	56	33	25	20	15
M-8	51	45	45	35	9	7
M-9	82	45	25	21	4	13
M-10	116	104	42	23	15	8

Appendix VI Fig. 32



Dye appearance time = 3.5 sec. Tube transit time =5.4 sec.

Curve area = 380 units. Half curve area = 190 units.

Mean transit time = (Appearance time-Tubing time) + Half Curve Area Time

= (7.5-5.4) + 5.4 = 7.5 sec.

Pulmonary Blood Volume = Flow times MTT $= \frac{250}{60}$ cc/sec $\times$ 7.5 = 31cc

Appendix VII Table XIII

Individual Pulmonary Artery Pressures (mm Hg) During Alternate Negative-Positive Pressure Ventilation in 8 Lobes (Equal Tidal Volumes)

	N	P	N	P	N	P	N	P
Expt. L-7	15	16	14	15	14	15	13	17
L-8	6	7	10	9	11	11	12	13
L-9	18	19	15	12	12	16	16	19
L-11	11	12	11	13	13	15	13	17
L-12	13	13	12	15	16	21	24	30
L-13	19	18	17	18	18	19	17	21
L-15	14	19	19	17	19	18	21	20
L-16	8	10	9	12	12	18	14	18

N = Negative

P = Positive

Appendix VIII Table XIV

Individual (Pulmonary Artery Pressure (mm Hg) - Pulmonary Venous Pressure)/Flow Values in Alternate Negative-Positive Pressure Ventilation in 8 Lobes

	N	P	N	P	N	P	N	P
Expt. L-7	13/250	14/250	12/250	13/250	12/250	13/250	4/250	15/250
L-8	5/250	8/250	7/250	9/250	9/250	10/250	11/250	13/250
L-9	16/255	17/255	13/255	10/255	10/255	14/255	14/255	17/255
L-11	9/298	10/298	9/298	11/298	11/298	13/298	11/298	15/298
L-12	11/315	11/315	10/315	13/315	14/315	19/315	22/315	28/315
L-13	17/346	16/346	15/346	16/346	16/346	17/346	15/346	19/346
L-15	17/225	17/225	15/225	17/225	16/225	19/225	18/225	19/225
L-16	6/435	8/435	7/435	10/435	10/435	16/435	12/435	16/435

Appendix IX Table XV

Individual Determinations of Pulmonary Vascular Resistance in Alternate Negative-Positive Pressure Ventilation of 8 Lobes

	N	P	N	P	N	P	N	P
Expt. L-7	4156	4476	3836	4156	3836	4156	3516	4795
L-8	1598	2557	2238	2877	2877	3197	3516	4156
L-9	5011	5331	4076	3133	3133	4388	4388	5331
L-11	2414	2685	2414	2949	2949	3485	2949	4020
L-12	2789	2789	2533	3301	3548	4819	5578	7105
L-13	3924	3692	3469	3692	3692	3924	3469	4388
L-15	6034	6042	5331	6042	5682	6745	6394	6745
L-16	1103	1471	1287	1838	1838	2941	2206	2941

$$\text{Pulmonary vascular resistance (dynes sec. cm}^{-5}\text{)} = \frac{(PA-PV) \times 1332}{Flow} \times 60$$

Appendix X

Individual Dynamic Compliance

Ratios [Tidal Volume (cc)/Ventilating Pressure (cm HOH)] During

Alternate Negative and Positive Pressure Ventilation in 8 Lobes

Expt. No.	Lobe Weight (g.)	N	P	N	P	N	P	N	P
L-7	37.6	70/6	70/7	50/5	50/6	90/5	90/8	65/6	65/9
L-8	54.0	75/6	70/6	95/6	90/7	90/6	85/8	80/5	75/6
L-9	48.9	105/4	105/8	65/6	70/6	50/4	50/5	65/6	70/6
L-11	53.1	95/5	90/7	85/5	90/7	90/6	95/7	95/6	100/8
L-12	65.2	110/5	110/8	120/5	120/10	90/5	100/8	70/5	75/13
L-13	74.4	100/5	95/8	115/5	115/9	90/6	90/8	90/5	85/7
L-15	51.4	85/5	85/6	65/6	60/5	95/6	90/7	60/5	60/5
L-16	76.8	170/5	170/8	165/5	170/9	145/5	140/8	155/5	150/9

APPENDIX XI

Students' t test was calculated using the cumulative mean difference (X_1) from 32 samples. This was compared to an expected mean (X_2) of 0, since for statistical purposes it was considered that the difference between positive and negative ventilation would be nil. The number of samples being compared in each case was 32 (t_1 and t_2). The standard deviation of each mean was taken as the S.D. of the cumulative mean differences.

1. Pulmonary vascular resistance

$x_1=570$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=370$

$S.D._2=370$

$t=6.06511$

$p<.005$

2. Compliance

$x_1=-9.5$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=8.0$

$S.D._2=8.0$

$t=4.67519$

$p<.005$

3. (a-v) CO_2

$x_{.1}=3.7$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=3.7$

$S.D._2=3.7$

$t = 3.93700$

$p<.005$

4. (v-a) O_2

$x_1=10.0$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=9.9$

$S.D._2=9.9$

$t = 4.30000$

$p<.005$

5. Dye appearance

$x_1=-0.6$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=0.7$

$S.D._2=0.7$

$t=3.38000$

$p<.005$

6. Pulm. Blood Vol.

$x_1=-2.5$

$x_2=0$

$t_1=32$

$t_2=32$

$S.D._1=4.9$

$S.D._2=4.9$

$t=2.00867$

$p<.05$

BIBLIOGRAPHY

1. TERASAKI, P.I., MICKEY, M.R., SINGAL, D.P., MUTTAL, K.K. and PATEL, R.: Serotyping for Homotransplantation XX. Selection of Recipients for Cadaver Donor Transplants. New Eng. J. Med. 279:1101, 1968.

2. BOGARDUS, G.M. and SCHLOSSER, R.J.: The Influence of Temperature Upon Ischemic Renal Damage. Surgery 39: 970, 1956.

3. LEVY, M.N.: Oxygen Consumption and Blood Flow in the Hypothermic Perfused Kidney. Amer. J. Physiol. 197: 1111, 1959.

4. SHERMAN, J.K.: Freezing and Freeze-drying of Bull Spermatozoa. Amer. J. Physiol. 190:281, 1957.

5. HUGGINS, C.E.: Preservation of Organized Tissues by Freezing. Fed. Proc. 24:5-190, 1965.

6. MANAX, W.G., BLOCH, J.H., LONGERBEAM, J.K. and LILLEHEI, R.C.: Successful 24 Hour In Vitro Preservation of Canine Kidneys by the Combined Use of Hyperbaric Oxygenation and Hypothermia. Surgery 56:275, 1964.

7. MANAX, W.G., EYAL, Z., BLOCK, J.H., LARGIADER, F., HIDALGO, F. and LILLEHEI, R.C.: Prolonged In Vitro Preservation of Whole Organs. 50th Clinical Congress of the American College of Surgeons, Chicago, Illinois, October 1964.

8. MANAX, W.G., BLOCH, J.H., EYAL, Z., LYONS, G.W. and LILLEHEI, R.C.: Whole Canine Organ Preservation. J.A.M.A. 196:105, 1966.

9. MANAX, W.G., BLOCH, J.H., EYAL, Z., LYONS, G.W. and LILLEHEI, R.C.: Hypothermia and Hyperbaria. J.A.M.A. 192:755, 1965.

10. LILLEHEI, R.C., MANAX, W.G., BLOCK, J.H., EYAL, Z., HIDALGO, F. and LONGERBEAM, J.K.: In Vitro Preservation of Whole Organs by Hypothermia and Hyperbaric Oxygenation. Cryobiology 1:181, 1964.

11. BLOCH, J.H., MANAX, W.G., EYAL, Z. and LILLEHEI, R.C.: Heart Preservation In Vitro with Hyperbaric Oyxgenation and Hypothermia. J. Thorac. & Cardiovasc. Surg. 48:969, 1964.

12. HANGAARD, N.: Effect of Oxygen Tensions on Enzymes: In Proceedings of Underwater Physiology Symposium. Ant. Acad. Sci. Pub. 337:8, 1955.

13. DICKENS, F.: The Toxic Effects of Oxygen on Brain Metabolism and on Tissue Enzymes; 2. Tissue Enzymes. Biochem. J. 40:171, 1946.

14. McBRIDE, C.M., HEALEY, J.E. Jr. and IBANEZ, M.L.: Effect of Extracorporeal Storage on Canine Kidneys. Tr. Am. Soc. Artif. Int. Organs 12:259, 1966.

15. BRETTSCHNEIDER, L., DALOZE, P.M. HUGUET, C., PORTER, K.A., GROTH, C.G., KASHIWAGI, N., HUTCHISON, D.E. and STARZL, T.E.: Use of Combined Preservation Techniques for Extended Storage of Orthotopic Liver Homografts. Surg. Gynec. & Obstet. 126:263, 1968.

16. ACKERMAN, J.R. and BARNARD, C.N.: Successful Storage of Kidneys. Brit. J. Surg. 53:525, 1966.

17. FEEMSTER, J.A. and LILLEHEI, R.C.: Hypothermic-Hyperbaric Pulsatile Perfusion for Preservation of the Canine Heart. Trans. Proc. 1:138, 1969.

18. WEBB, W.R., DODDS, R.P., UNAL, M.O., KAROW, A.M. COOK, W.A. and DANIEL, C.R.: Suspended Animation of the Heart with Metabolic Inhibitors. Ann. Surg. 164:343, 1966.

19. CLUNIE, G.J.A., LINDSEY, E.S. and REEMTSMA, K.: Mercurial Diuretic Protection of the Ischemic Kidney in the Rat. Surg. Forum 17:239, 1966.

20. WEISSMAN, G.: Labilization and Stabilization of Lysosomes. Fed. Proc. 23:1038, 1964.

21. CARREL, A. and LINDBERGH, C.A.: In, The Culture of Organs. Paul B. Hoeber, Inc., New York, p. 205.

22. BELZER, F.O., PARK, H.Y. and VETTO, R.M.: Factors Influencing Renal Blood Flow in Isolated Perfusion. Surg. Forum 15:222, 1964.

23. KESTENS, P.J., FARRELLY, J.A. and McDERMOTT, W.V.: A Technique of Isolation and Perfusion of the Canine Liver. Surgery 50:196, 1961.

24. BOXALL, T.A., HIROSE, S. and EISEMAN, B.: The Ex Vivo Perfused Spleen. Surg. Forum 17:260, 1966.

25. AUSTEN, W.G. and McLAUGHLIN, E.D.: In Vitro Small Bowel Perfusion. Surg. Forum 16:359, 1965.

26. DOBELL, A.R., MITRI, M., GALVA, R., SARKOZY, E. and MURPHY, D.R.: Biologic Evaluation of Blood After Prolonged Recirculation Through Film and Membrane Oxygenators. Ann. Surg. 161:617, 1965.

27. LEE, W.H. Jr., KRUMHAAR, D., FONKALSRUD, E.W., SCHJEIDE, O.A. and MALONEY, J.V. Jr.: Denaturation of Plasma Proteins as a Cause of Morbidity and Death After Intracardiac Operaticns. Surgery 50:29, 1961.

28. HUGGINS, C.E.: Survival of Hamsters After 4 Hours of Cardiac Arrest at 0°C. Surg. Forum 12:413, 1961.

29. CALLAGHAN, P.B., LISTER, J., PATON, B.C. and SWAN, H.: Effects of Varying Carbon Dioxide Tensions on the Oxyhemoglobin Dissociation Curves Under Hypothermic Conditions. Ann. Surg. 154:903, 1961.

30. HUMPHRIES, A.L. Jr., RUSSEL, R., GREGORY, J., CARTER, R.H. and MORETZ, W.H.: Hypothermic Perfusion of the Canine Kidney for 48 Hours Followed by Reimplantation. Am. Surgeon 30:748, 1964.

31. BELZER, F., ASHBY, B.S. and DUNPHY, J.E.: 24 Hour and 72 Hour Preservation of Canine Kidneys. Lancet 2:536, 1967.

32. HUMPHRIES, A.L. Jr., RUSSEL, R., STODDARD, L.D. and MORETZ, W.H.: Successful Five Day Kidney Preservation. Perfusion with Hypothermic Diluted Plasma. Invest. Urol. 5:609, 1968.

33. BLOCH, J.H., MANAX, W.G., EYAL, Z. and LILLEHEI, R.C.: Organ Perfusion Before Transplantation. Fed. Proc. 23:237, 1964.

34. ACKERMANN, J.R.W. and BARNARD, C.N.: Successful Storage of Kidneys. Brit. J. Surg. 53:525, 1966.

35. ANGELL, W.W. and SHUMWAY, N.E.: Resuscitative Storage of the Cadaver Heart Transplant. Surg. Forum 17:224, 1966.

36. MARTIN, H.N.: On a Method of Isolating a Mammalian Heart. Science 2:228, 1881.

37. JACOBI, C.: Ein Beitrag zur Technik der Kunstlichen Durchblutang Uberlebender Organe. Arch. Exptl. Pathol. Phamakol. 36:330, 1895.

38. STARLING, E.H. and VERNEY, E.B.: The Secretion of Urine in Studies on the Isolated Kidney. Proc. R. Soc. Ser. B. Biol. Sc. London, 97:321, 1925.

39. HEMINGWAY, A.: Some Observations on Perfusion of Isolated Kidney by Pump. J. Physiol., London, 71:201, 1931.

40. ALCOCK, P., BERRY, J.L., DALY, I. de Burgh, and NARAYANA, B.: The Action on Perfused Lungs of Drugs Injected into the Bronchial Vascular System. Quart. of Exper. Physiol. 26:14, 1937.

41. DALY, I. de Burgh.: Observations on the Blood-Perfused Lungs of the Dog, Guinea-Pig, and Macacus Rhesus, With Special Reference to "Spontaneous" Lung Movements. Quart. J. Exper. Physiol. 28:357, 1938.

42. DUKE, H.N.: Pulmonary Vasomotor Responses of Isolated Perfused Cat Lungs to Anoxia and Hypercapnia. Quart. J. Exper. Physiol. 36:75, 1951.

43. NISELL, O.: Effects of Oxygen and Carbon Dioxide on the Circulation of Isolated and Perfused Lungs of the Cat. Acta. Physiol. Scand. 16:121, 1949.

44. RILEY, R.L., COURNAND, A. and DONALD, K.W.: Analysis of Factors Affecting Partial Pressures of Oxygen and Carbon Dioxide in Gas and Blood of Lungs: Methods. Am. J. Appl. Physiol. 4:102, 1951-2.

45. THOMAS, L.J., GRIFFO, Z.J. and ROOS, A.: Effect of Negative Pressure Inflation of the Lung on Pulmonary Vascular Resistance. J. Appl. Physiol. 16:451, 1961.

46. BANISTER, J. and TORRANCE, R.W.: The Effects of the Tracheal Pressure Upon Flow: Pressure Relationships in The Vascular Bed of Isolated Lungs. Quart. J. Exper. Physiol. 45:352, 1960.

47. PERMUTT, S., BROMBERGER-BARNEA, B. and BANE, H.N.: Alveolar Pressure, Pulmonary Venous Pressure and the Vascular Waterfall. Med. Thorac. 19:239, 1962.

48. WEST, J.B., DOLLERY, C.T. and NAIMARK, A.: Distribution of Blood Flow in Isolated Lung: Relation to Vascular and Alveolar Pressures. J. Appl. Physicl. 19: 713, 1964.

49. POTTS, W.J., RIKER, W.L., DeBORD, R. and ANDREWS, C.E.: An Experimental Study of Respiration Maintained by Homologous Lungs. J. Lab and Clin. Med. 38:281, 1951.

50. WESOLOWSKI, S.A., FISHER, J.H. and WELCH, C.S.: Heart-Lung Bypass Using Pumps and Isolated Homologous Lungs. Surg. Gynec. & Obstet. 95:762, 1952.

51. CRISP, N.W., CAMPBELL, G.S. and BROWN, E.B.: Studies on Perfusion of Human Blood Through the Isolated Dog Lung. Surg. Forum 6:286, 1955.

52. MUSTARD, W.T. and CHUTE, A.L.: A Surgical Approach to Transposition of the Great Vessels with Extracorporeal Circuit. Surgery 36:39, 1954.

53. COHEN, M., WARDEN, H.E. and LILLEHEI, C.W.: Physiologic and Metabolic Changes During Autogenous Lobe Oxygenation with Total Cardiac By-Pass Employing the Azygos Flow Principle. Surg. Gynec. & Obstet. 98: 523, 1954.

54. PIERPONT, H. and BLADES, B.: Lung Perfusion With Chemotherapeutic Agents. J. Thorac. & Cardiovasc. Surg. 39: 159, 1960.

55. PIERPONT, H. and DAVIDSON, C.: Lung Perfusion. J.A.M.A. 179:121, 1962.

56. EISEMAN, B.: Extracorporeal Assist - Liver, Spleen and Lung. Scand. J. Gastroent. 1:241, 1966.

57. EISEMAN, B., BRYANT, L. and WALTUCH, T.: Metabolism of Vasomotor Agents by the Isolated Perfused Lung. J. Thorac. & Cardiovasc. Surg. 48:798, 1964.

58. BRYANT, L.R., EISEMAN, B. and AVERY, M.: Studies of the Porcine Lung as an Oxygenator for Human Blood. J. Thorac. & Cardiovasc. Surg. 55:255, 1968.

59. RATLIFF, J.L.: Extracorporeal Biologic Oxygenation for Prolonged Total Respiratory Support. J. Thorac. & Cardiovasc. Surg. 55:686, 1968.

60. VEITH, F.J., DEYSINE, M., NEHLSEN, S.L. and KARL, R.C.: Preservation of Pulmonary Function, Hemodynamics and Morphology in Isolated Perfused Canine Lungs. J. Thorac. & Cardiovasc. Surg. 52:437, 1966.

61. VEITH, F.J., HAGSTROM, W.C., NEHLSEN, S.L., KARL, R.C. and DEYSINE, M.: Functional, Hemodynamic, and Anatomic Changes in Isolated Perfused Dog Lungs: The Importance of Perfusate Characteristics. Ann. Surg. 165:267, 1967.

62. VEITH, F.J., DEYSINE, M., NEHLSEN, S.L., PANNOSSIAN, A. and HAGSTROM, J.W.C.: Pulmonary Changes Common to Isolated Lung Perfusion, Venovenous Bypass and Total Cardiopulmonary Bypass. Surg. Gynec. & Obstet. 125: 1047, 1967.

63. AWAD, J.A., LOU, W., LEMIEUX, J.M. and BEAULIEU, M.: A Technique for the Perfusion of Pulmonary Lobes for Prolonged Periods. A Report of an Experimental Study. Can. J. Surg. 8:100, 1965.

64. AWAD, J.A., CHENARD, J., LOU, W., BEAULIEU, M. and LEMIEUX, J.M.: The Effect of Interruption of the Bronchial Circulation and the Addition of Cortisone and Low Molecular Weight Dextran to Blood Perfusate During Pulmonary Perfusion in Dogs. Can. J. Surg. 10:94, 1967.

65. AWAD, J.A., CHENARD, J., LEMIEUX, J.M. and LOU, W.: Evaluation of Various Fluids for the Perfusion of the Canine Pulmonary Lobes. Dis. Chest 48:269, 1965.

66. BROWNLEE, R.T., COUVES, C.M., DRITSAS, K.G. and KOWALEWSKI, K.: Metabolic Alterations in Pulmonary Tissue Preserved for Transplantation. Can. J. Surg. 11:237, 1968.

67. LUNDE, P.K.M., HAUGE, A. and WAALER, B.A.: Experimental Oedema in Isolated Perfused Rabbit Lungs. Conference of Pulmonary Circulation, Carsten Muller, Editor, Scan. Univ. Books, Oslo, 1966.

68. OTTO, T.J., TRENKNER, M., STOPCZYK, A., GAWDZINSKI, M. and CHELSTOWSKA, B.: Perfusion and Ventilation of Isolated Canine Lungs. Thorax 23:645, 1968.

69. MILLER, D.R. and KUENZIG, M.C.: Pulmonary Changes Following Normothermic and Profound Hypothermic Perfusion in Dogs. J. Thorac. & Cardiovasc. Surg. 56: 717, 1968.

70. HOMATAS, J., BRYANT, L. and EISEMAN, B.: Time Limits of Cadaver Lung Viability. J. Thorac. & Cardiovasc. Surg. 56:132, 1968.

71. FISK, R.L., BROWNLEE, R.T., BROWN, D.R., MacFARLANE, D.F., BUDNEY, D., DRITSAS, K.G., KOWALEWSKI, K. and COUVES, C.M.: Perfusion of Isolated Organs for Prolonged Functional Preservation. In: Organ Perfusion and Preservation, edited by Norman, J.C., New York, 1968, Appleton-Century-Crofts, Inc.

72. FISK, R.L., SYMES, J.F., ALDRIDGE, L.L. and COUVES, C.M.: Improved Perfusion of Functioning Isolated Lungs Based on Normal Pulmonary Hemodynamics. J. Thorac. & Cardiovasc. Surg. 58:231, 1969.

73. DRITSAS, K.G., BROWN, D. and COUVES, C.M.: Perfusion-Ventilation of the Isolated Canine Lung: Hemodynamic Considerations. Surgery 65:611, 1969.

74. COUVES, C.M., DRITSAS, K.G., BROWN, D.R., and FISK, R.L.: The Role of Venous Pressure on the Capillary Circulation. Scientific Exhibit, American College of Surgeons, 1966.

75. CLARKE, D.B.: Perfusion Techniques in the Preservation of the Isolated Canine Lung. Thorax 24:342, 1969.

76. CLARKE, C.P., KAHN, D.R., DUFEK, J.H. and SLOAN, H.: The Effects of Nonpulsatile Blood Flow on Canine Lungs. Ann. Thorac. Surg. 6:450, 1968.

77. MALONEY, J.E., BERGEL, D.H., GLAZIER, J.M.B., HUGHES, M.B. and WEST, J.B.: Effect of Pulsatile Pulmonary Artery Pressure on Distribution of Blood Flow in Isolated Lung. Respir. Physiol. 4:154, 1968.

78. TROWELL, O.A.: The Histology of the Isolated Perfused Lung. Quart. J. Exper. Physiol. 32:203, 1943-44.

79. AWAD, J.A., LEMIEUX, J.M., LOU, W.: Pulmonary Complications Following Perfusion of the Lungs. J. Thorac. & Cardiovasc. Surg. 51:767, 1966.

80. HENRY, J.N. McARDLE, A.H., SCOTT, H.J. and GURD, F.N.: A Study of the Acute and Chronic Respiratory Pathophysiology of Hemorrhagic Shock. J. Thorac. & Cardiovasc. Surg. 54:666, 1967.

81. NASH, G., BLENNERHASSETT, J.B. and PONTOPPIDAN, H.: Pulmonary Lesions Associated with Oxygen Therapy and Artificial Ventilation. New Eng. J. Med. 276:368, 1967.

82. ROWLAND, R. and NEWMAN, C.G.H.: Pulmonary Complications of Oxygen Therapy. J. Clin. Path. 22:192, 1969.

83. MILLER, D.R., DIXON, H.K., and AKIMOTO, S.: Pulmonary Embolectomy for Lethal Emboli in Dogs. J. Thorac. & Cariovasc. Surg. 53:477, 1967.

84. DALLDORF, F.G., CARNEY, C.N., RACKLEY, C.E. and RANEY, R.B. Jr.: Pulmonary Capillary Thrombosis in Septicemia Due to Gram-Positive Bacteria. J.A.M.A. 206: 583, 1968.

85. BLADES, B., BEATTIE, E.J. Jr., HILL, R.P. and THISTLEWAITE, R.: Ischemia of the Lung. Ann. Surg. 136:56, 1952.

86. LONG, D.M., FOLKMAN, J., NEPTUNE, E.M. and SUDDUTH, H.C.: Pulmonary Airway Changes Resulting From Ischemia of the Pulmonary Artery. Surg. Forum 13:164, 1962.

87. BORRIE, J. and LICHLER, I.: Lung Transplantation: Technical Problems. Thorax 19:383, 1964.

88. HARDY, J.D., ERASLAN, S. and DALTON, M.L. Jr.: Auto-transplantation and Homotransplantation of the Lung. Further Studies. J. Thorac. & Cardiovasc. Surg. 46: 606, 1963.

89. BLUMENSTOCK, D.A., HECHTMAN, H.B., and COLLINS, J.A.: Preservation of the Canine Lung. J. Thorac. & Cardio-vasc. Surg. 44:771, 1962.

90. LARGIADER, F., MANAX, W.G., LYONS, G.W. and LILLEHEI, R.C.: In Vitro Preservation of Canine Heart and Lung. Arch. Surg. 91:801, 1965.

91. BLUMENSTOCK, D.A., LEMPERT, N. and MORGADO, F.: Pre-servation of the Canine Lung In Vitro for 24 Hours With the Use of Hypothermia and Hyperbaric Oxygen. J. Thorac. & Cardiovasc. Surg. 50:769, 1965.

92. GARZON, A.A., CHENG, C., LICHTENSTEIN, S. and KARLSON, K.E.: Functional Evaluation of the Lung Preserved for Twenty Four Hours. Surg. Forum 17:208, 1966.

93. ROSS, C.A. and ALVES, R.F.: Preservation of Canine Lung for Re-implantation Using Hypothermia and Hyper-baric Oxygen. Thorax 24:336, 1969.

94. THEODORIDES, T., WEBB, W.R., NAKAE, S. and SUGG, W.L.: Prolonged Survival of the Anoxic Lung with Metabolic Inhibitors in Normothermic and Hypothermic Conditions. Ann. Thorac. Surg. 5:411, 1968.

95. GARZON, A.A., CHENG, C., PANGAN, J. and KARLSON, K.E.: Hypothermic Hyperbaric Lung Preservation for 24 Hours with Reimplantation. J. Thorac. & Cardiovasc. Surg. 55:546, 1968.

96. GARZON, A.A., MINKOWITZ, S., CHENG, C. and KARLSON, K.E.: Twenty-four Hour Lung Storage. In: Organ Per-fusion and Preservation, edited by Norman, J.C. New York, 1968, Appleton-Century-Crofts, Inc. p. 665.

97. Handbook of Respiration, National Academy of Sciences, National Research Council, Dittmer, D.S. and Grebe, R.E., Editors. Philadelphia, 1958, W.B. Saunders Co.

98. BROWN, B.L. and NAGLE, S.C.: Preservation of Mammalian Cells in a Chemically Defined Medium and Dimethylsulfo-xide. Science 149:1266, 1965.

99. ROBERTSON, R.D. and JACOB, S.W.: The Preservation of Intact Organs. Adv. Surg. 3:75, 1968.

100. DALY, I de B. and WAALER, B.A.: Bronchial Circulation and Pulmonary Vascular Resistance in Isolated Perfused Lung Lobes. J. Physiol., London, 153:14P, 1960.

101. THELMO, W., BECKER, N.H., HAGSTROM, J.W.C. and VEITH, F.J.: Sequential Light and Electron Microscopy of Isolated Perfused Lungs. Ann. Surg. 171:171, 1970.

102. SMITH, C.W., LEHAN, P.H. and MONKS, J.J.: Cardiopulmonary Manifestations with High Oxygen Tensions at Atmospheric Pressure. J. Appl. Physiol. 18:849, 1963.

103. FLETCHER, G. and BUNKER, J.P.: Physiologic Basis of Prolonged Artificial Ventilation. Ann. Rev. Med. 17: 473, 1966.

104. GUYTON, A.C., ROSS, J.M., CARRIER, O. Jr. and WALKER, J.R.: Evidence for Tissue Oxygen Demand as the Major Factor Causing Autoregulation. Circ. Res. 14 & 15:1-60, 1964, Suppl. I.

105. HOHRST, H.J.: Determination of Tissue Lactate, Ph.D. Thesis, Universitat Marburg, 1960.

106. HOHORST, H.J.: Determination of Tissue Pyruvate. Biochem. 2. 332:18, 1959.

107. PARKHURST, A.S., BURKHALTER, A. and COHN, H.C. Jr.: A Method for the Fluorometric Assay of Histamine in Tissues. J. Pharm. & Exper. Therap. 127:182, 1959.

108. WAALKES, T.P.: The Determination of Serotonin in Human Blood. J. Lab. & Clin. Med. 53:824, 1959.

109. WEBER, K.C. and VISSCHER, M.B.: Metabolism of the Isolated Canine Lung. Amer. J. Physiol. 217:1044, 1969.

110. HUCKABEE, W.E.: Relationships of Pyruvate and Lactate during Anaerobic Metabolism. I. Effects of Infusion of Pyruvate or Glucose and of Hyperventilation. J. Clin. Invest. 37:244, 1958.

111. WEISSMAN, G.: Lysosomes. New Eng. J. Med. 273: 1084, 1965 and 273:1143, 1965.

112. TAPLEY, D.F., KIMBERG, D.V. and BUCHANAN, J.L.: The Mitrochondrion. New Eng. J. Med. 276:1124, 1967.

113. WOOLSTENHOLME, G.E.W. and O'CONNOR, C.M.: Ciba Symposium Foundation on Histamine. London: Churchill, 1956.

114. Handbook of Physiology: A Critical, Comprehensive Presentation of Physiological Knowledge and Concepts. Section 2, Edited by Hamilton, W.F. and Dow, P. Amer. Physiol. Soc., Washington, 1965, p. 1059.

115. STORSTEIN, O., CALABRESI, M., NIMS, R.G. and GRAY, F.D. Jr.: The Effect of Histamine on the Pulmonary Circulation in Man. Yale J. Biol. Med. 32:197, 1959a.

116. DOUGLAS, W.W.: Histamine and Antihistamines. In: The Pharmacological Basis of Therapeutics, Edited by Goodman, L.S. and Gilman, A. Third edition, 1965.

117. DePASQUALE, N.P., SANCHEZ, G., BURCH, G.E. and QUIROZ, A.C.: Effect of Bradykinin on the Pulmonary Vascular Bed of the Intact Dog. Amer. Heart J. 78:802, 1969.

118. SAID, S.I.: Vasoactive Substances in the Lung. In: Current Research in Chronic Obstructive Lung Disease. Proceedings of Tenth Aspen Emphysema Conference, 1967, p. 223.

119. JUHLIN, L.: Reactions to Infusion of a Histamine Liberator. Arch. Derm. 88:771, 1963.

120. GADDUM, J.H.: Antagonism Between LSD and 5-Hydroxytryptamine. J. Physiol. London, 121:15, 1953.

121. BRYANT, L.R., EISEMAN, B. and AVERY, M.: Studies of the Porcine Lung as an Oxygenator for Human Blood. J. Thorac. & Cardiovasc. Surg. 55:255, 1968.

122. HOMATAS, J., BRYANT, L. and EISEMAN, B.: Time Limits of Cadaver Lung Viability. J. Thorac. & Cardiovasc. Surg. 56:132, 1968.

123. NASH, G., BLENNERHASSETT, J.B. and PONTOPPIDAN, H.: Pulmonary Lesions Associated with Oxygen Therapy and Artificial Ventilation. New Eng. J. Med. 276:368, 1967.

124. BERGMAN, N.A.: Distribution of Inspired Gas During Anesthesia and Artificial Ventilation. J. Appl. Physiol. 18:1085, 1963.

125. WHITTENBERGER, J.L.: Artificial Respiration. Physiol. Reviews. 35:611, 1955.

126. HUBAY, C.A., BRECHER, G.A. and CLEMENT, F.L.: Etiological Factors Affecting Pulmonary Artery Flow with Controlled Respiration. Surgery 38:215, 1955.

127. BURTON, A.C. and PATEL, D.J.: Effect on Pulmonary Vascular Resistance of Inflation of the Rabbit Lungs. J. Appl. Physiol. 12:239, 1958.

128. GERST, P.H.: The Effects of Positive Pressure Lung Inflation on Pulmonary Vascular Dynamics. J. Thorac. & Cardiovasc. Surg. 42:607, 1961.

129. ZIERLER, K.L.: Theoretical Basis of Indicator-Dilution Methods for Measuring Flow and Volume. Circ. Res. 10:393, 1962.

130. PONTOPPIDAN, H., HEDLEY-WHYTE, J., BENDIREN, H.H., LAVER, M.B. and RADFORD, E.P. Jr.: Ventilation and Oxygen Requirements During Prolonged Artificial Ventilation in Patients with Respiratory Failure. New. Eng. J. Med. 273:401, 1965.

131. ZIELINSKI, J.: Oxygen Tension in Arterial Blood During Assisted Ventilation in Patients with Chronic Respiratory Failure. Respiration 27:162, 1970.

132. STERN, L., RAMOS, A.D., OUTERBRIDGE, E.W. and BEAUDRY, P.H.: Negative Pressure Artificial Respiration Used in Respiratory Failure of the Newborn. Can. Med. Ass. J. 102:595, 1970.

133. LINSAS, L.S., LEVISON, H. and SWYER, P.R.: Negative Pressure Artificial Respiration: Use in Treatment of Respiratory Distress Syndrome of the Newborn. Can. Med. Ass. J. 102:602, 1970.